OIPE JC116
SEP 20 2004
PATENT & TRADEMARK OFFICE

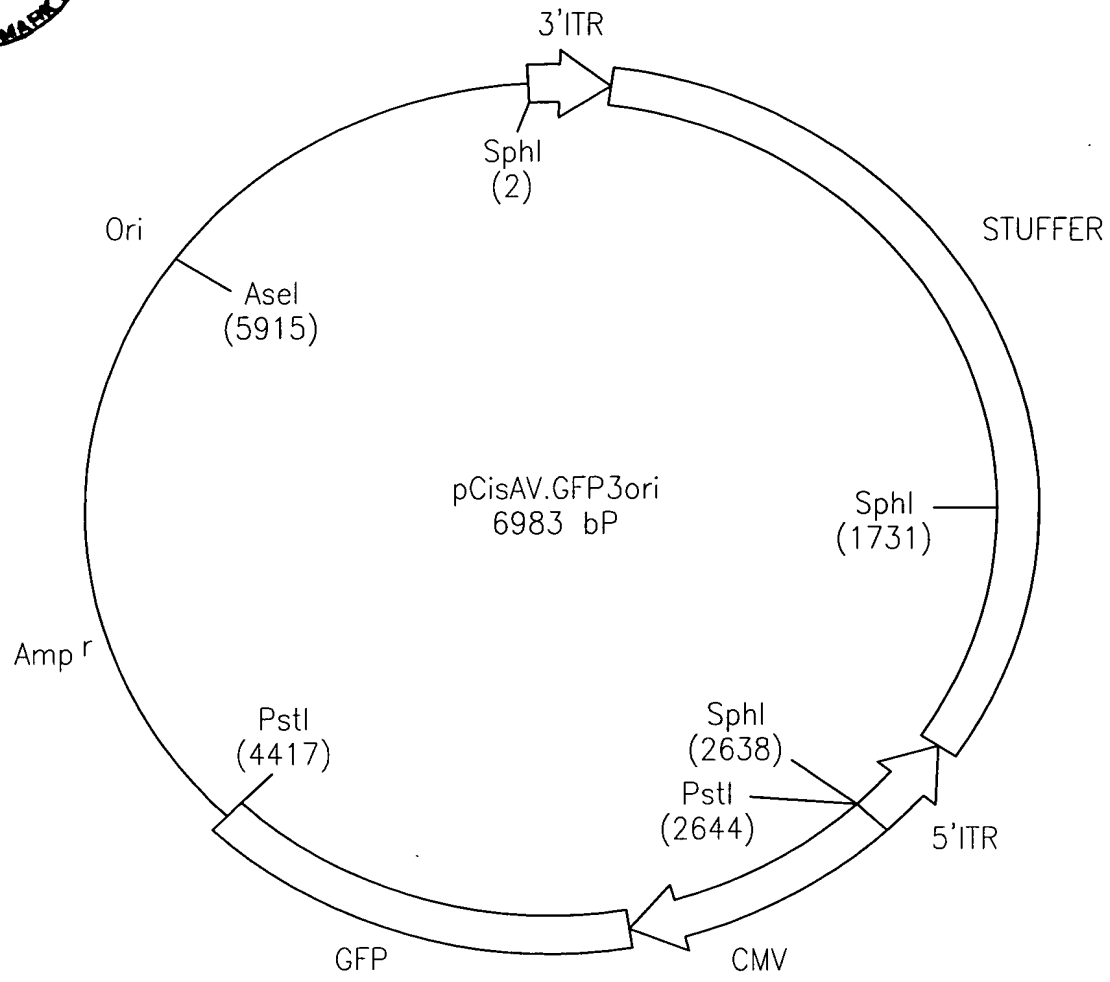



FIG. 1A

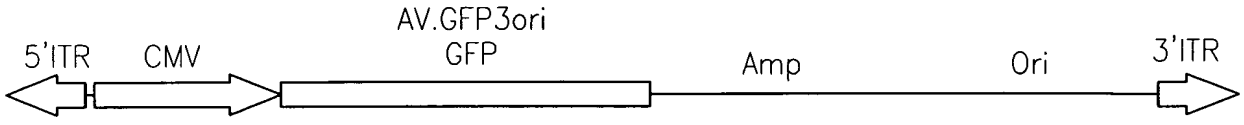



FIG. 1B

BEST AVAILABLE COPY

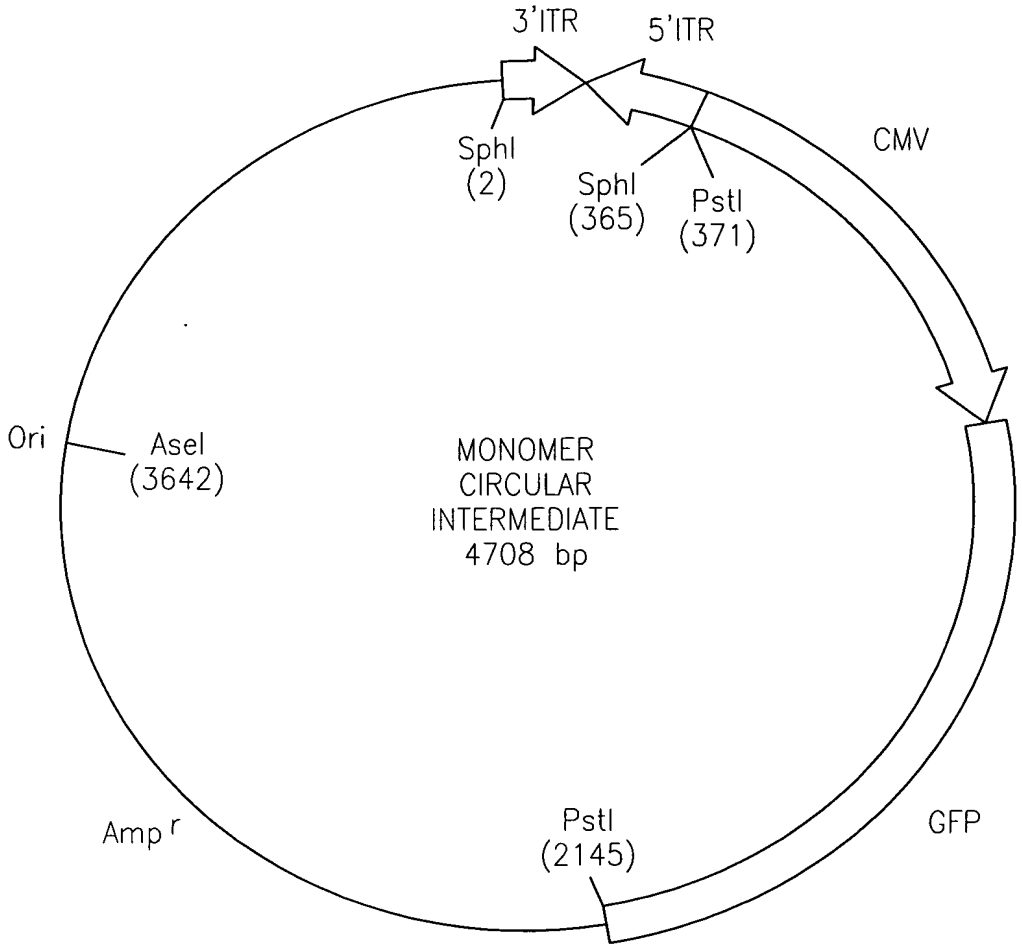

3'ITR
5'ITR
CMV
SphI
(2)
SphI
(365)
PstI
(371)
Ori
AseI
(3642)
MONOMER
CIRCULAR
INTERMEDIATE
4708 bp
Amp r
PstI
(2145)
GFP

FIG. 1C

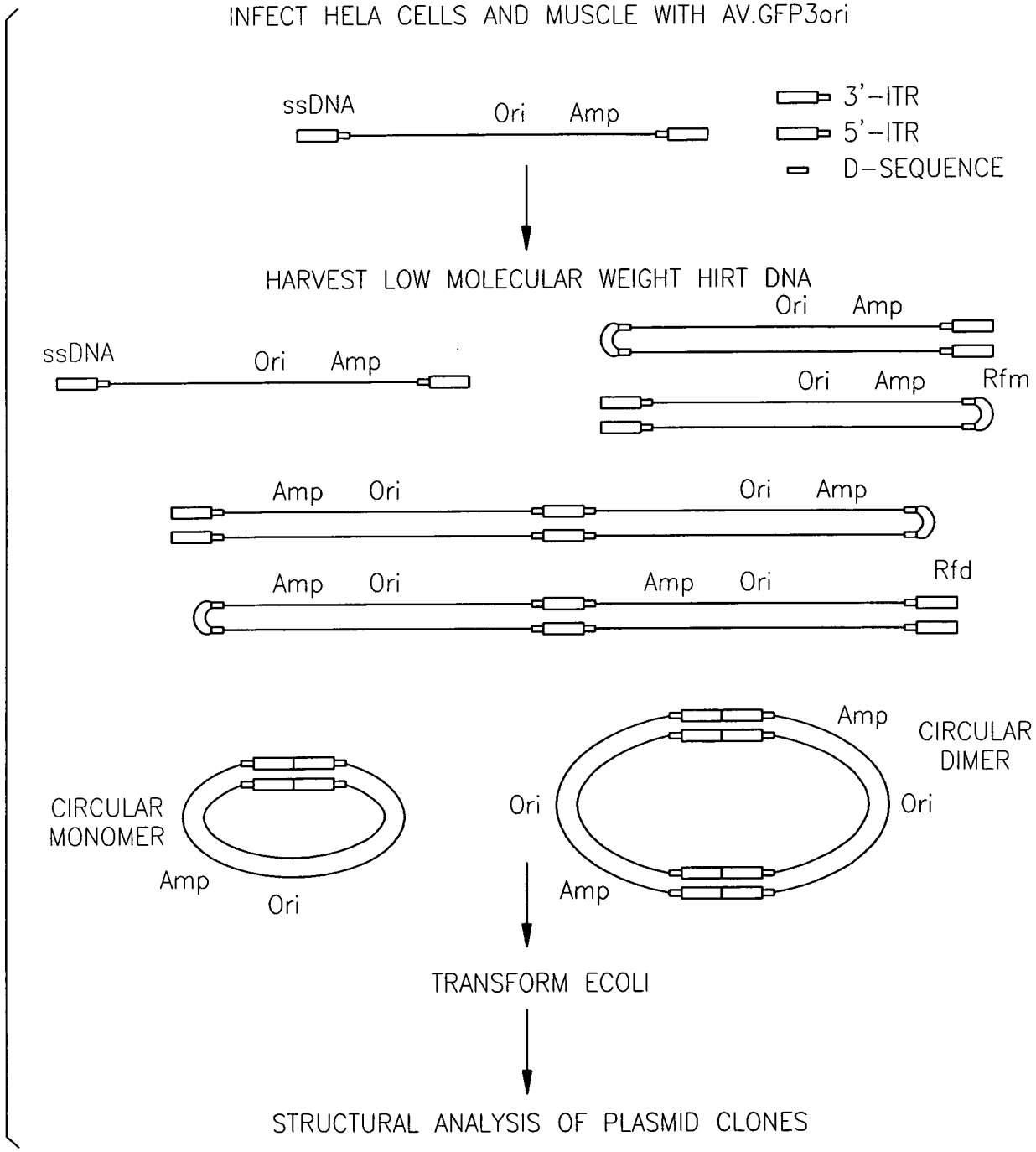

INFECT HELA CELLS AND MUSCLE WITH AV.GFP3ori
ssDNA
Ori
Amp
3'-ITR
5'-ITR
D-SEQUENCE
HARVEST LOW MOLECULAR WEIGHT HIRT DNA
ssDNA
Ori
Amp
Ori
Amp
Ori
Amp
Rfm
Amp
Ori
Ori
Amp
Amp
Ori
Amp
Ori
Rfd
CIRCULAR MONOMER
Amp
Ori
Amp
Ori
Ori
Amp
CIRCULAR DIMER
TRANSFORM ECOLI
STRUCTURAL ANALYSIS OF PLASMID CLONES

FIG. 1D

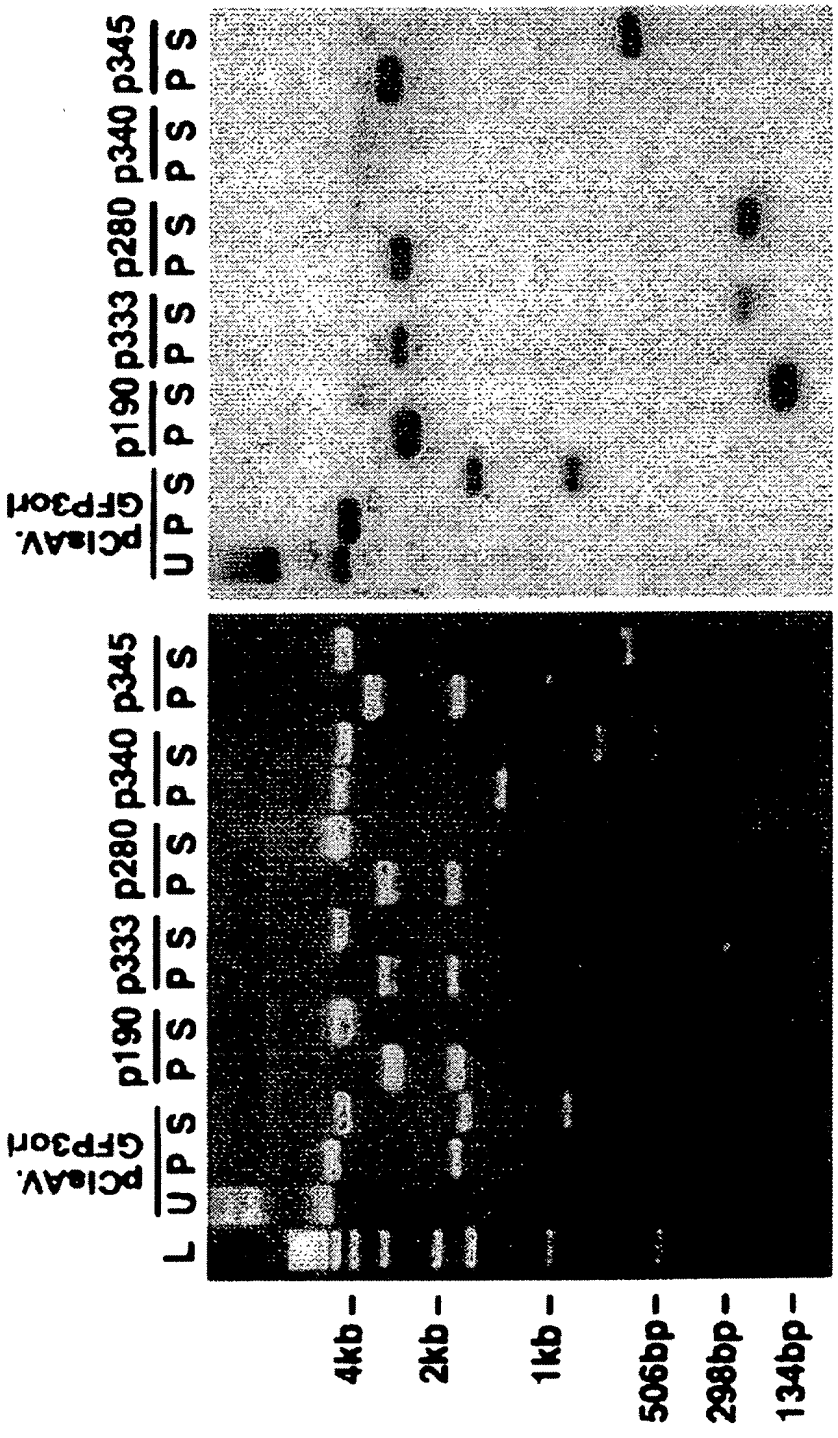

L
U
P
S
GClaAV.GFP3on
p190
p333
p280
p340
p345
4kb
2kb
1kb
506bp
298bp
134bp

FIG. 2A

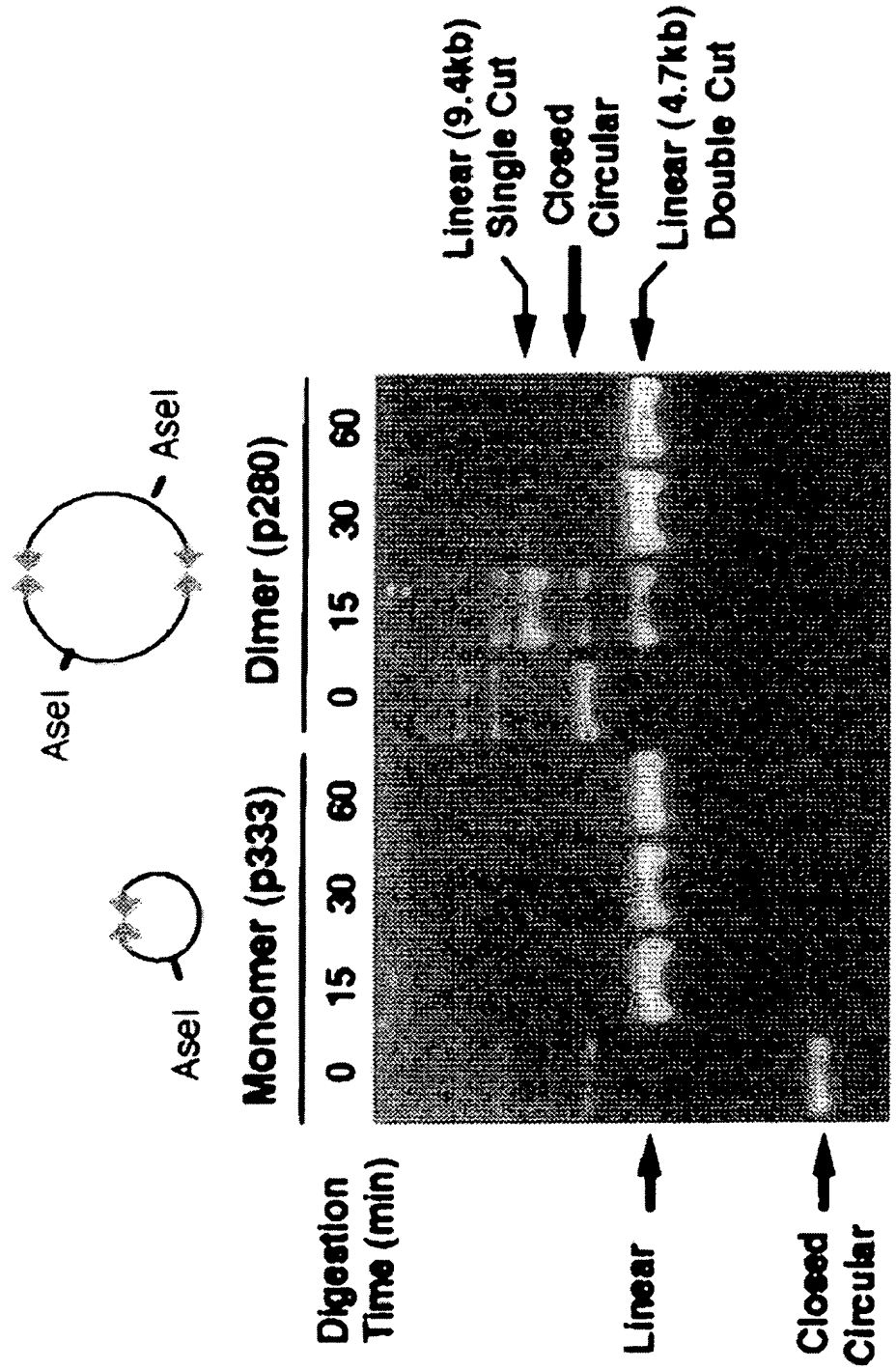

AseI
AseI
AseI
Monomer (p333)
Dimer (p280)
0 15 30 60 0 15 30 60
Digestion Time (min)
Linear (9.4kb) Single Cut
Closed Circular
Linear (4.7kb) Double Cut
Linear
Closed Circular

FIG. 2B

```
                    10         20         30         40         50         60         70
5 <->3  ITR  CGGGGGTCGT TGGGCGGTCA GCCAGGCGGG CCATTTACCG TAAGTTATGT AACGACTGCA GGCATGCAAG
        p79  ---------- ---------- ----GGCGGG CCATTTACCG TAAGTTATGT ggCGACTGCA GGCATGCAAG
        P80  ---------- ---------- --------GG CCATTTACCG TAAGTTATGT AACGACTGCA GGCATGCAAG
        P81  ---------- ---------- --------GG CCATTTACCG TAAGTTATGT ggCGACTGCA GGCATGCAAG
        P86  ---------- ---------- --------GG CCATTTACCG TAAGTTATGT AACGACTGCA GGCATGCAAG
        P87  ---------- ---------- --------GG CCATTTACCG TAAGTTATGT AACGACTGCA GGCATGCAAG
        P88  ---------- ---------- ---------G CCATTTACCG TAAGTTATGT AACGACTGCA GGCATGCAAG

                   80         90        100        110        120        130        140
5 <->3  ITR  CTCGAATTCA TCGGTAGATA AGTAGCATGG CGGGTTAATC ATTAACTACA AGGAACCCCT AGTGATGGAG
        p79  CTCGAATTCA TCGGTAGATA AGTAGCATGG CGGGTTAATC ATTgcCTACA AaGAgCCCCT AGTGATGGAG
        P80  CTCGAATTCA TCGGTAGATA AGTAGCATGG CGGGTTAATC ATTAACTACA AGGAACCCCT AGTGATGGAG
        P81  CTCGAATTCA TCGGTAGATA AGTAGCATGG CGGGTTAATC ATTgcCTACA AaGAgCCCCT AGTGATGGA-
        P86  CTCGAATTCA TCGGTAGATA AGTAGCATGG CGGGTTAATC ATTAACTACA AGGAACCCCT AGTGATGGAG
        P87  CTCGAATTCA TCGGTAGATA AGTAGCATGG CGGGTTAcTC ATTgcCTACA AaGAgCCCCT AGTGATGGAa
        P88  CTCGAATTCA TCGGTAGATA AGTAGCATGG CGGGTTAATC ATTgcCTACA AaGAgCCCCT AGTGATGGAG

                  150        160        170        180        190        200        210
5 <->3  ITR  TTGGCCACTC CCTCTCTGCG CGCTCGCTCG CTCGCTGAGG CCGGGCGACC AAAGGTCGCC CGACGCCCGG
        p79  TgGGCCACTC CCTCTCTTCG C--------- ---------- ---------- ---------- ----------
        P80  TTGGCCACTC CCTCTCTGCG CGCTCGCTCG CTCGCTCAGG CCGGGCAACC AAAGGTCGCC CGACGCCC--
        P81  ---------- ---------- ---------- ---------- ---------- ---------- ----------
        P86  TTGGCCACTC CCTCTCTGCG CGCTCGCTCG CTCGCTGAGG CCGCCC---- ---------- ----------
        P87  TTGG------ ---------- ---------- ---------- ---------- ---------- ----------
        P88  TTGGCCACTC CCTCTCTGCG CGCTCGCTCG CTgG------ ---------- ---------- ----------
```

FIG. 2C

```
                  220        230        240        250        260        270        280
5 <->3  ITR  GCTTTGCCCG GGCGGCCTCA GTGAGCGAGC GAGCGCGCAG CTGCGCGCTC GCTCGCTCAC TGAGGCCGCC
        p79  ---------- ---------- ---------- ---------- ---------- ---------- ----------
        P80  ---------- ---------- ---------- ---------- ---------- ---------- ----------
        P81  ---------- ---------- ---------- ---------- ---------- ---------- ----------
        P86  ---------- ---------- ---------- ---------- ---------- ---------- ----------
        P87  ---------- ---------- ---------- ---------- ---------- ---------- ----------
        P88  ---------- ---------- ---------- ---------- ---------- ---------- ----------

                  290        300        310        320        330        340        350
5 <->3   ITR  CGGGCAAAGC CCGGGCGTCG GGCGACCTTT GGTCGCCCGG CCTCAGCGAG CGAGCGAGCG CGCAGAGAGG
        p79  ---------- ---------- ---------- ---------- ---------- ----CGAGCG CGCAGAGAGG
        P80  ---------- ---------- ---------- ----GCCCGG CCTCAGCGAG CGAGCGAGCG CGCAGAGAGG
        P81  ---------- ---------- ---------- ----GCCCGG CCTCAcCGAG CGAGCGAGCG CGCAGAGAGG
        P86  ---------- ---------- ---------- -------CGG CCTCAGCGAG CGAGCGAGCG CGCAGAGAGG
        P87  ---------- ---------- ---------- ---------- ---------- ---------- ----------
        P88  ---------- ---------- ---------- ----GCCCGG CCTCAGCGAG CGAGCGAGCG CGCAGAGAGG

                  360        370        380        390        400        410        420
5 <->3   ITR  GAGTGGCCAA CTCCATCACT AGGGGTTCCT TGTAGTTAAT GATTAACCCG CCATGCTACT TATCTACAGC
        p79  GAGTGGCCAA CTCCcTCACT AGGGGTTCCT gGcAGTTAAT GATTAACCCG CCATGCTACT TATCTACAGC
        P80  GAGTGGCCAA CTCCATCACT AGGGGTTCCT TGTAGTTAAT GATTAACCCG CCATGCTACT TATCTACAGC
        P81  GAGTGGCCAA CTCCATCACT AGGGGTTCCT TGTAGTTAAT GATTAACCCG CCATGCTACT TATCTACAGC
        P86  GAcTGGCCAA CTCCATCACT AGGGGTTCCT TGTAGTTAAT GATTAACCCG CCATGCTACT TATCTACAGC
        P87  ---------- ---------- ---------- -------AAT GATTcACCCt CCATGCTACT TATCTACAGC
        P88  GAGTGGCCAA CTCCATCACT AGGGGTTCCT gGcAGTTAAT GATTAACCCG CCATGCTACT TATCTACAGC
```

FIG. 2D

```
                    430        440        450        460        470        480        490
5 <->3   ITR  TTGCATGCAT GTGAGCAAAA GGCCAGCAAA AGGCCAGGAA CCGTAAAAAG GCCGCGTTGC TGGCGTTTTT
        p79   TTGCATGCAT GTGAGCAAAA GGCCAGCAAA AGGCCAGGAA CCGTAAAAAG GCCGCGTTG- ----------
        P80   TTGCATGCAT GTGAGCAAAA GGCCAGCAAA AGGCCAGGAA CCGTAAAAAG GCCGCGTTG- ----------
        P81   TTGCATGCAT GTGAGCAAAA GGCCAGCAAA AGGCCAGGAA CCGTAAAAAG GCCGCGTTG- ----------
        P86   TTGCATGCAT GTGAGCAAAA GGCCAGCAAA AGGCCAGGAA CCGTAAAAAG GCCGC----- ----------
        P87   TTGCATGCAT GTGAGCAAAA GGCCAGCAAA AGGCCAGGAA CCGTAAAAAG GCCGCGTTG- ----------
        P88   TTGCATGCAT GTGAGCAAAA GGCCAGCAAA AGGCCAGGAA CCGTAAAAAG GCCGCGTTG- ----------

                    500    505
5 <->3   ITR  CCATAGGCTC CGCCC
        p79   ---------- -----
        P80   ---------- -----
        P81   ---------- -----
        P86   ---------- -----
        P87   ---------- -----
        P88   ---------- -----
```

FIG. 2E

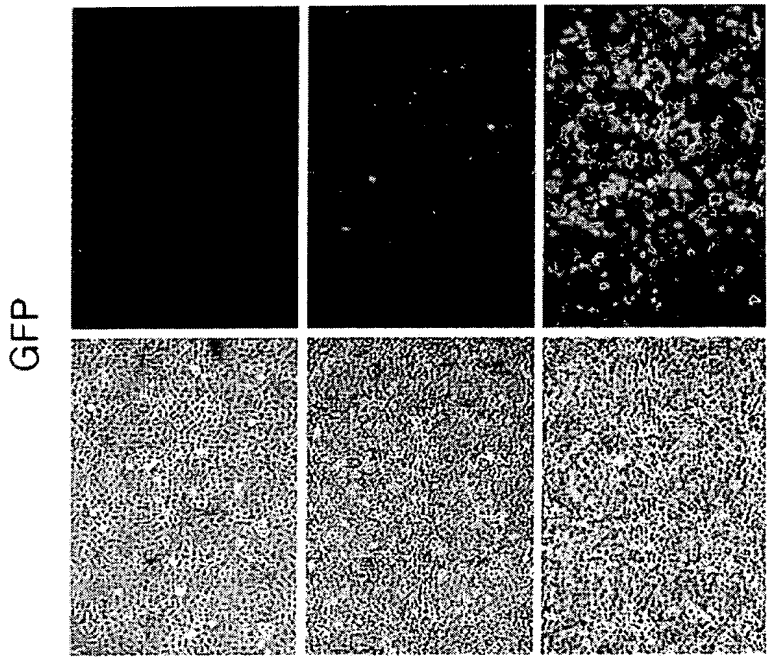



FIG. 3B

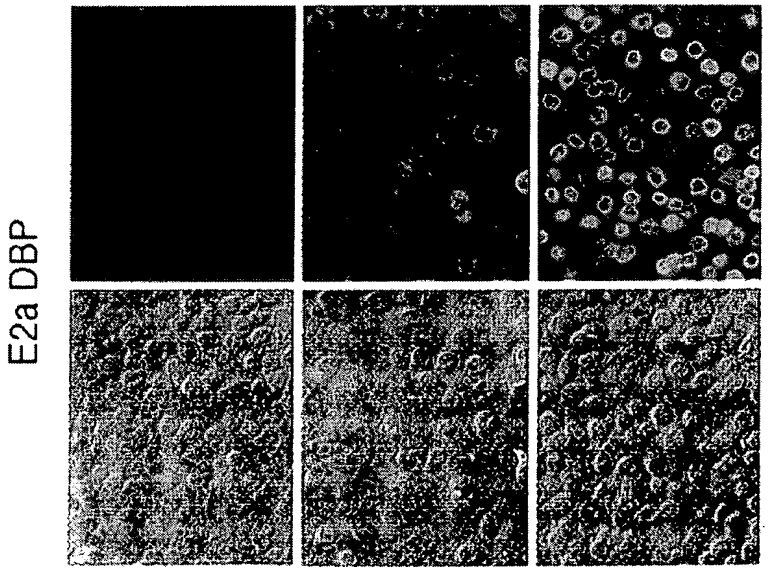
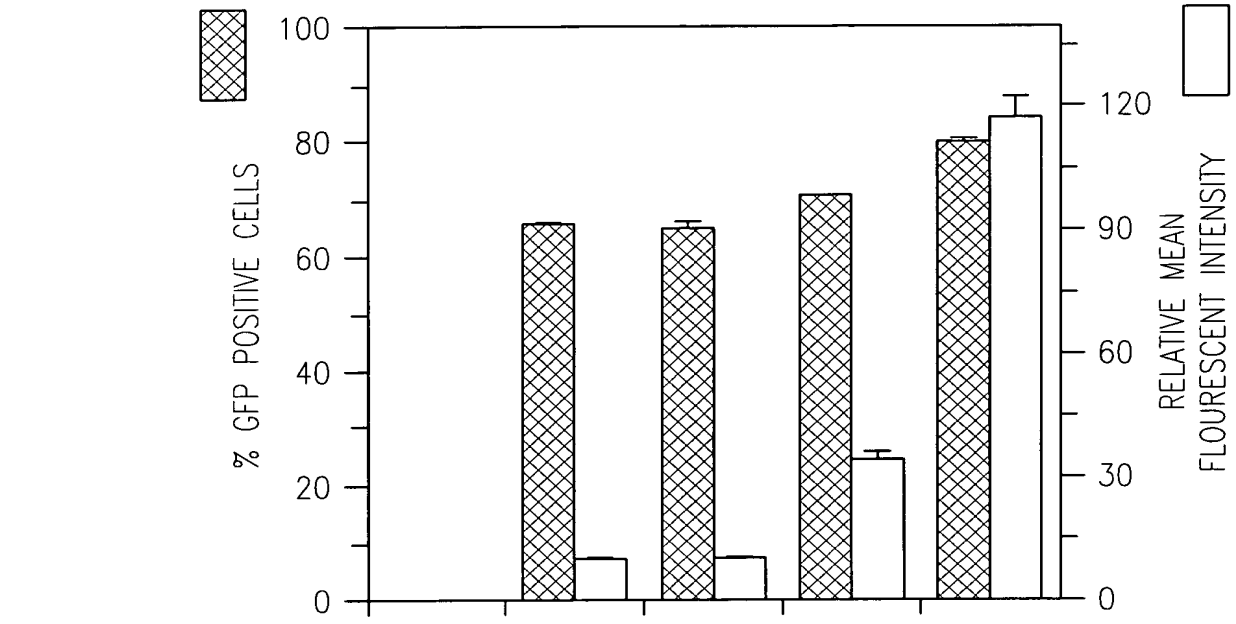



FIG. 3A




| | | | | | |
|---|---|---|---|---|---|
| AAV MOI (PART/CELL) | 0 | 1000 | 1000 | 1000 | 1000 |
| Ad.lacZ MOI (PART/CELL) | 0 | 0 | 500 | 5000 | 10000 |
| GFP CFU (6 DYS) | 0 | 2 | 5 | 31 | CPE |

FIG. 3C

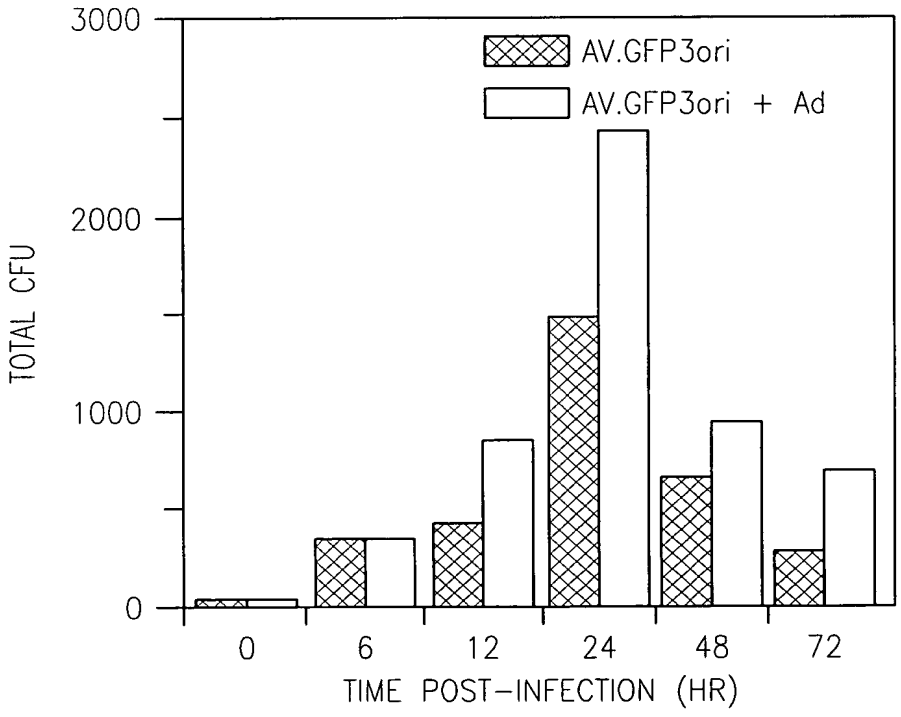



FIG. 3D

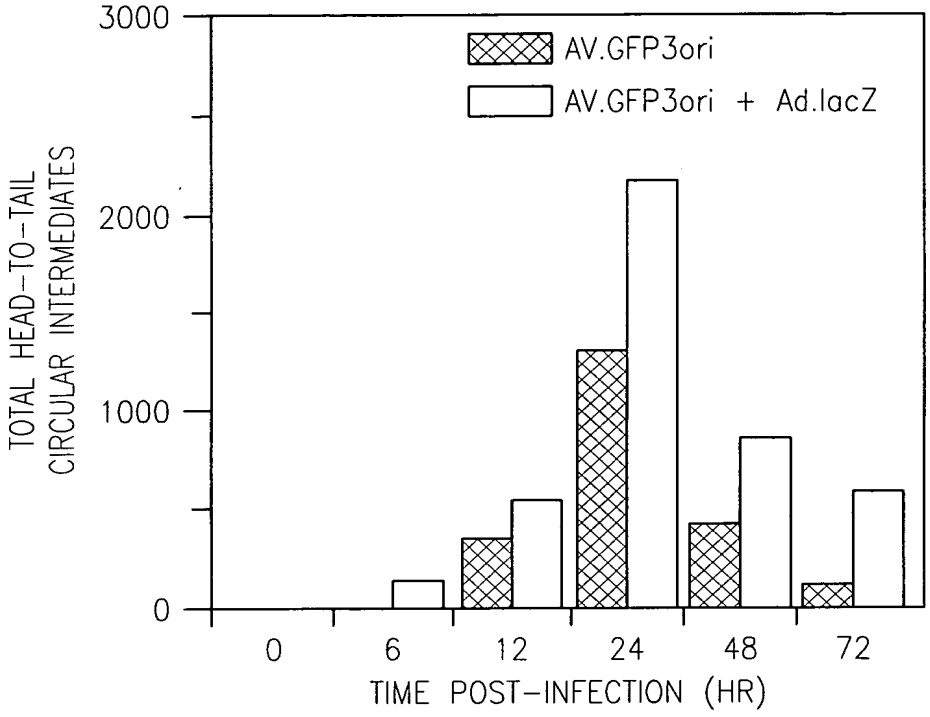

3000
2000
1000
0
TOTAL HEAD-TO-TAIL
CIRCULAR INTERMEDIATES
AV.GFP3ori
AV.GFP3ori + Ad.lacZ
0
6
12
24
48
72
TIME POST-INFECTION (HR)

FIG. 3E

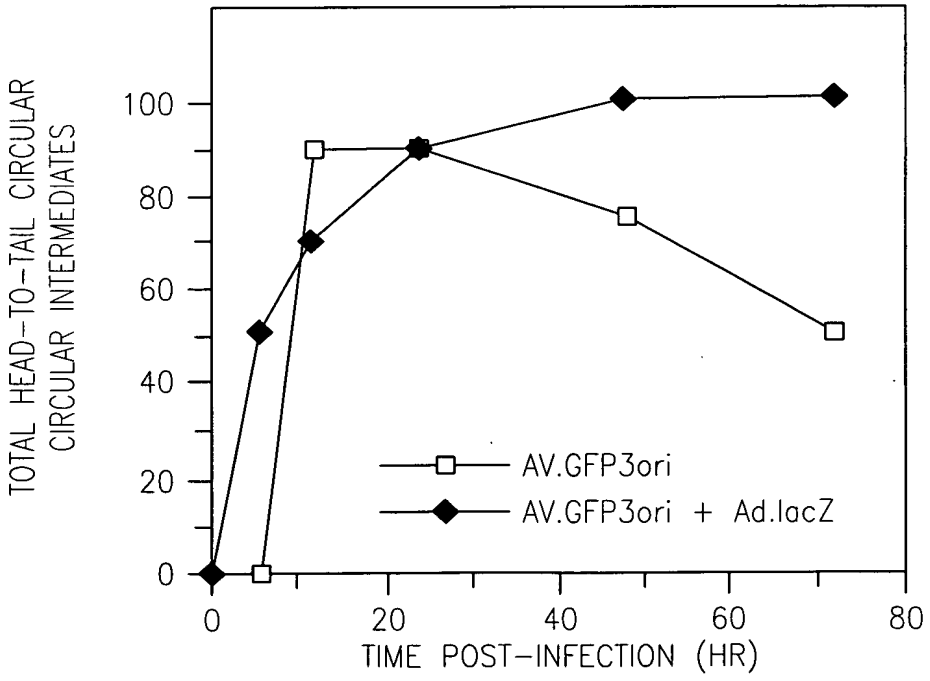

100
80
60
40
20
0
TOTAL HEAD-TO-TAIL CIRCULAR
CIRCULAR INTERMEDIATES
AV.GFP3ori
AV.GFP3ori + Ad.lacZ
0
20
40
60
80
TIME POST-INFECTION (HR)

FIG. 3F

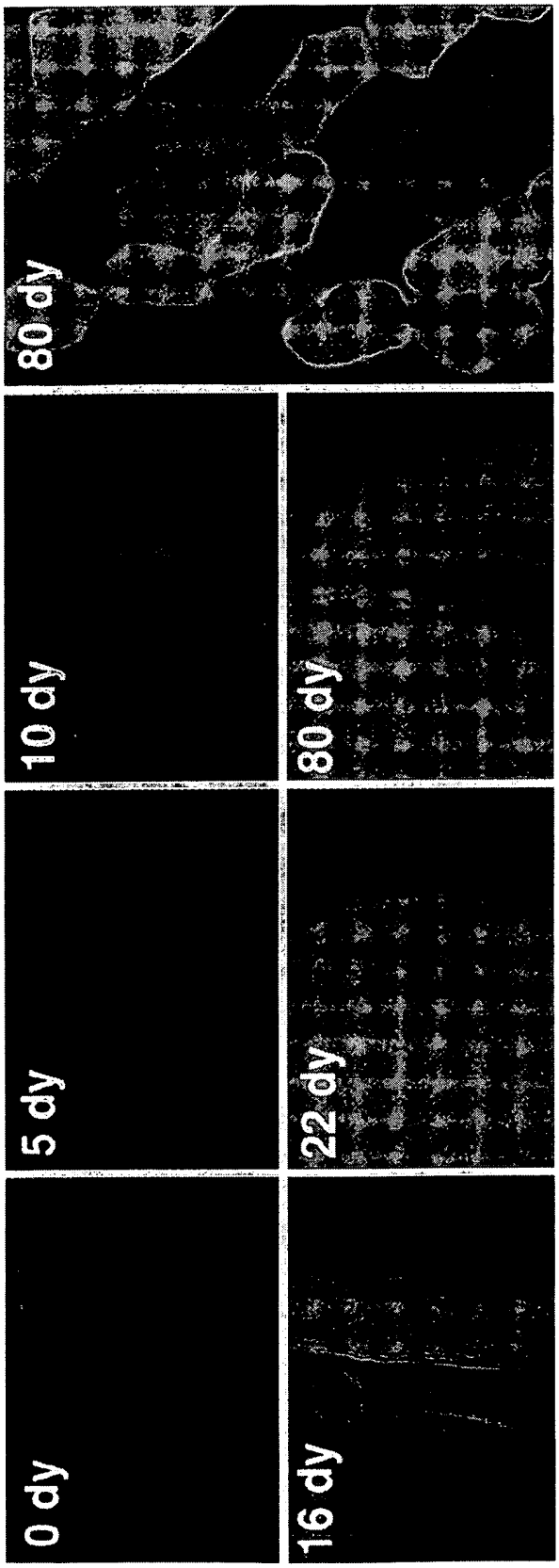



FIG. 4A

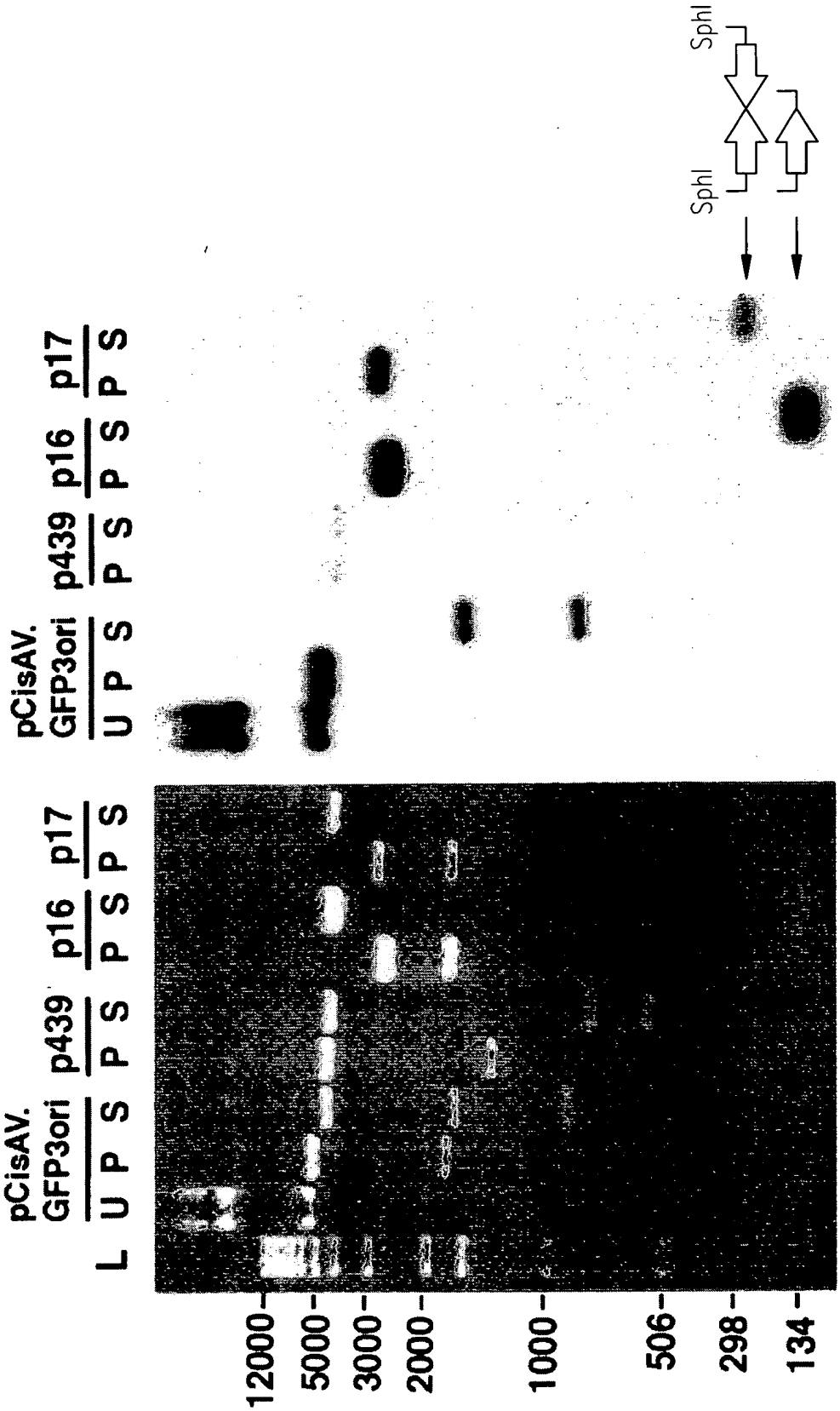

pCisAV.
GFP3ori
L U P S
p439
P S
p16
P S
p17
P S
12000
5000
3000
2000
1000
506
298
134
pCisAV.
GFP3ori
U P S
p439
P S
p16
P S
p17
P S
SphI
SphI

FIG. 4B

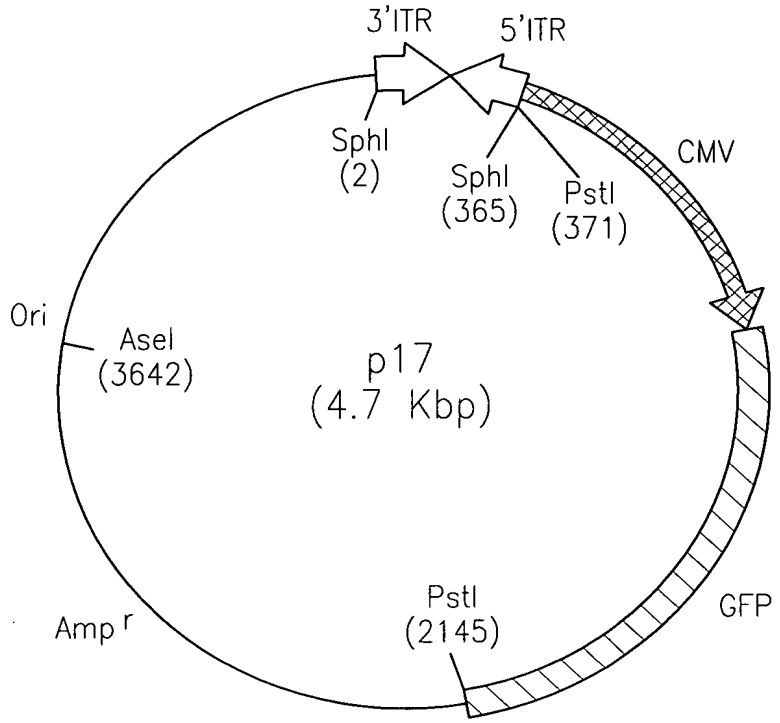

3'ITR
5'ITR
SphI
(2)
SphI
(365)
PstI
(371)
CMV
Ori
AseI
(3642)
p17
(4.7 Kbp)
Amp r
PstI
(2145)
GFP

FIG. 4C

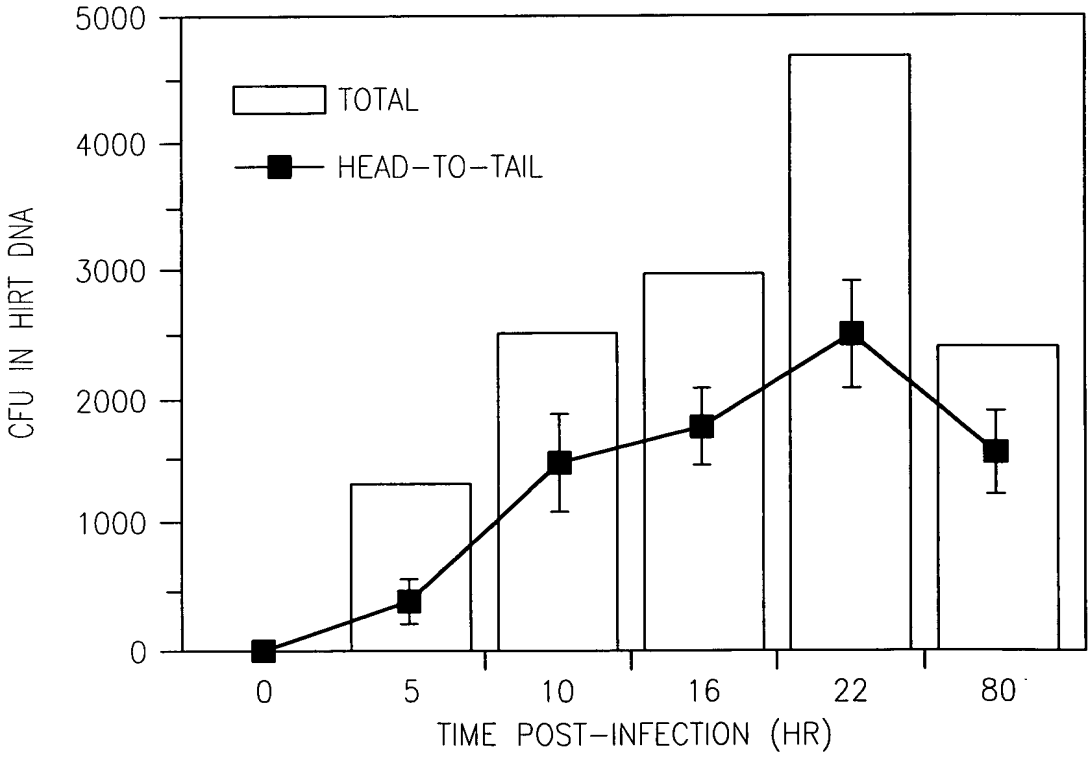

5000
4000
3000
2000
1000
0
TOTAL
HEAD-TO-TAIL
CFU IN HIRT DNA
0
5
10
16
22
80
TIME POST-INFECTION (HR)

FIG. 5A

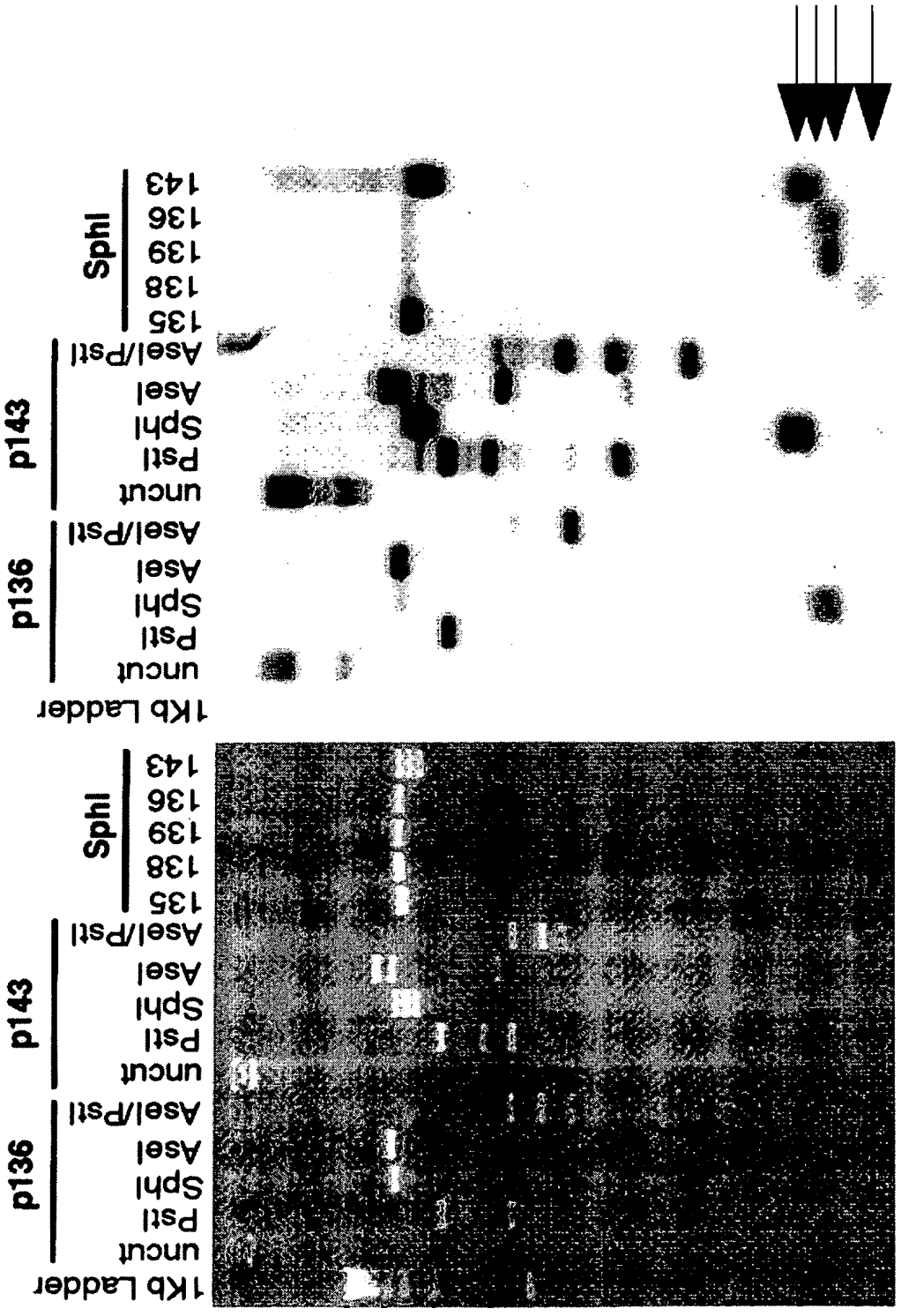

1Kb Ladder
p136
uncut
PstI
SphI
AseI
AseI/PstI
p143
uncut
PstI
SphI
AseI
AseI/PstI
SphI
135
138
139
136
143

FIG. 5B

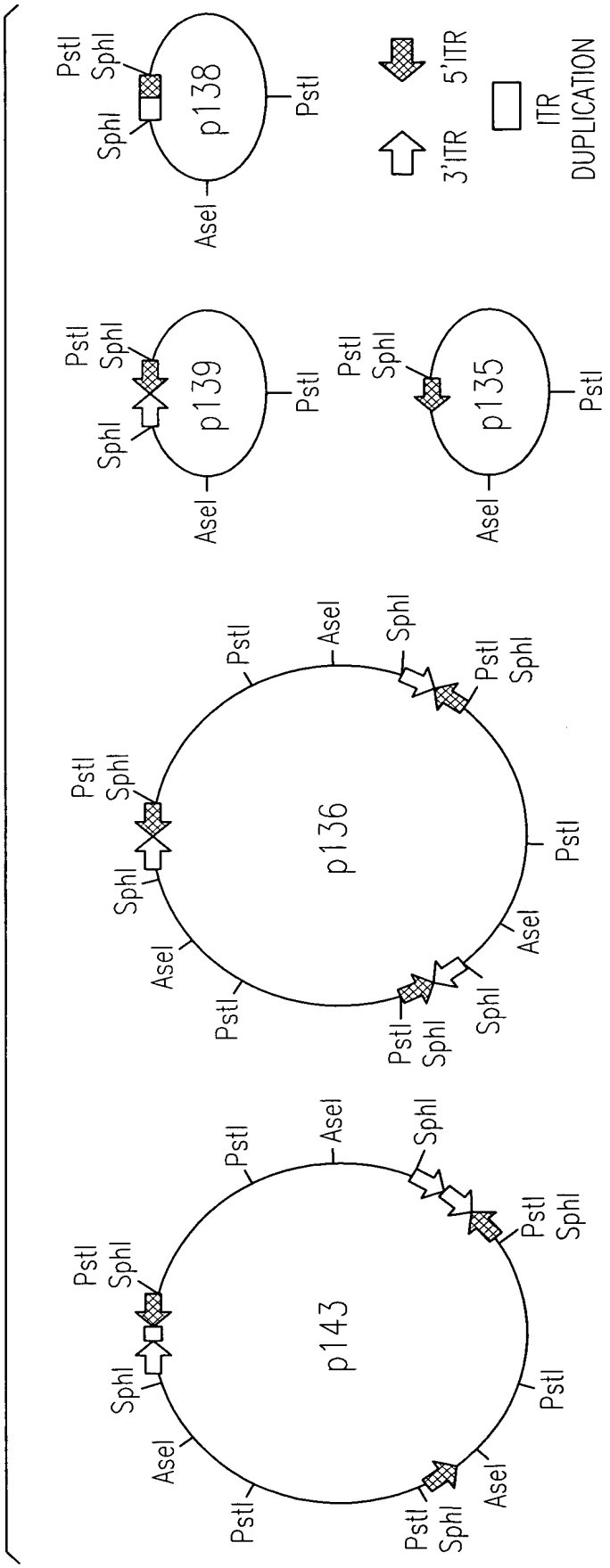

p138
p139
p135
p136
p143
PstI
SphI
AseI
3'ITR
5'ITR
ITR
DUPLICATION

FIG. 5C

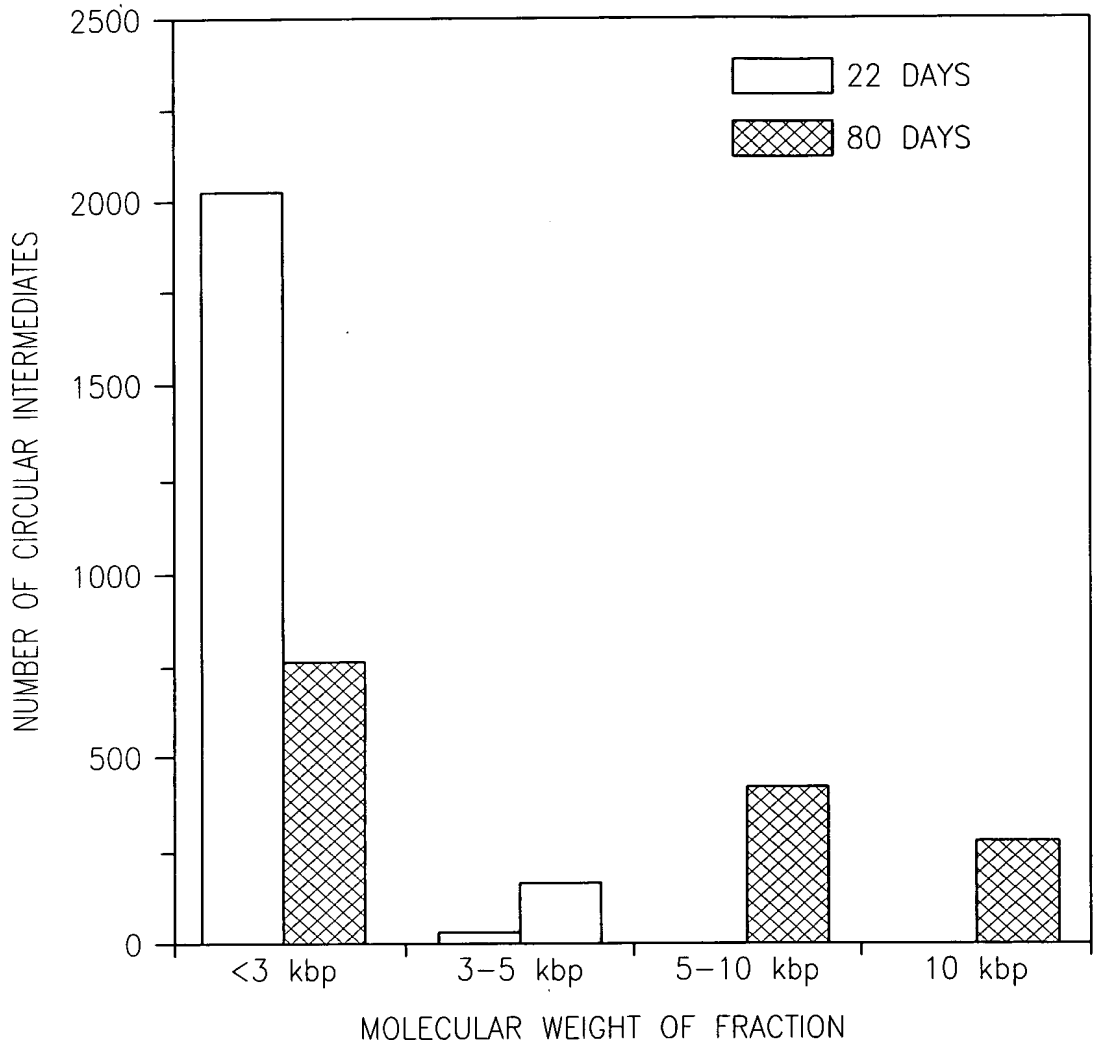

2500
2000
1500
1000
500
0
NUMBER OF CIRCULAR INTERMEDIATES
22 DAYS
80 DAYS
<3 kbp
3-5 kbp
5-10 kbp
10 kbp
MOLECULAR WEIGHT OF FRACTION

FIG. 6

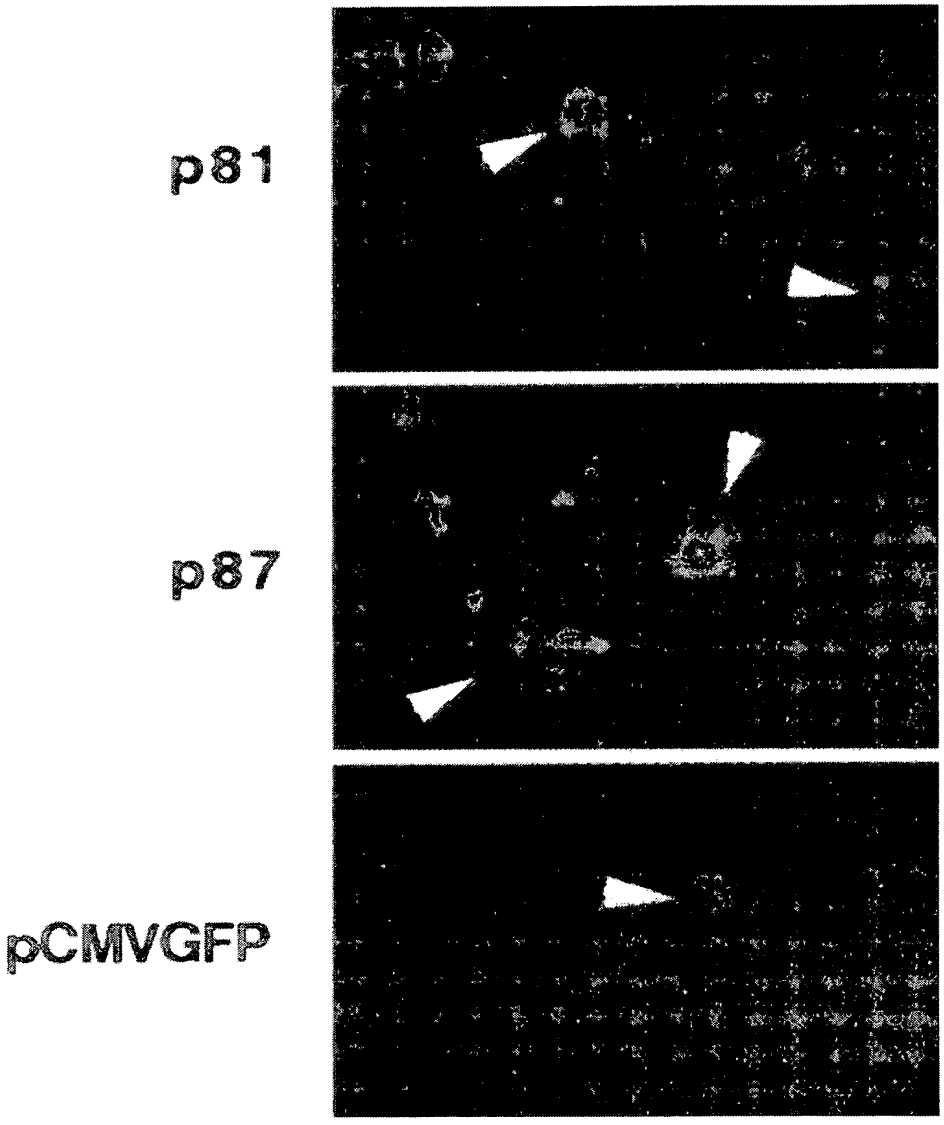



FIG. 7A

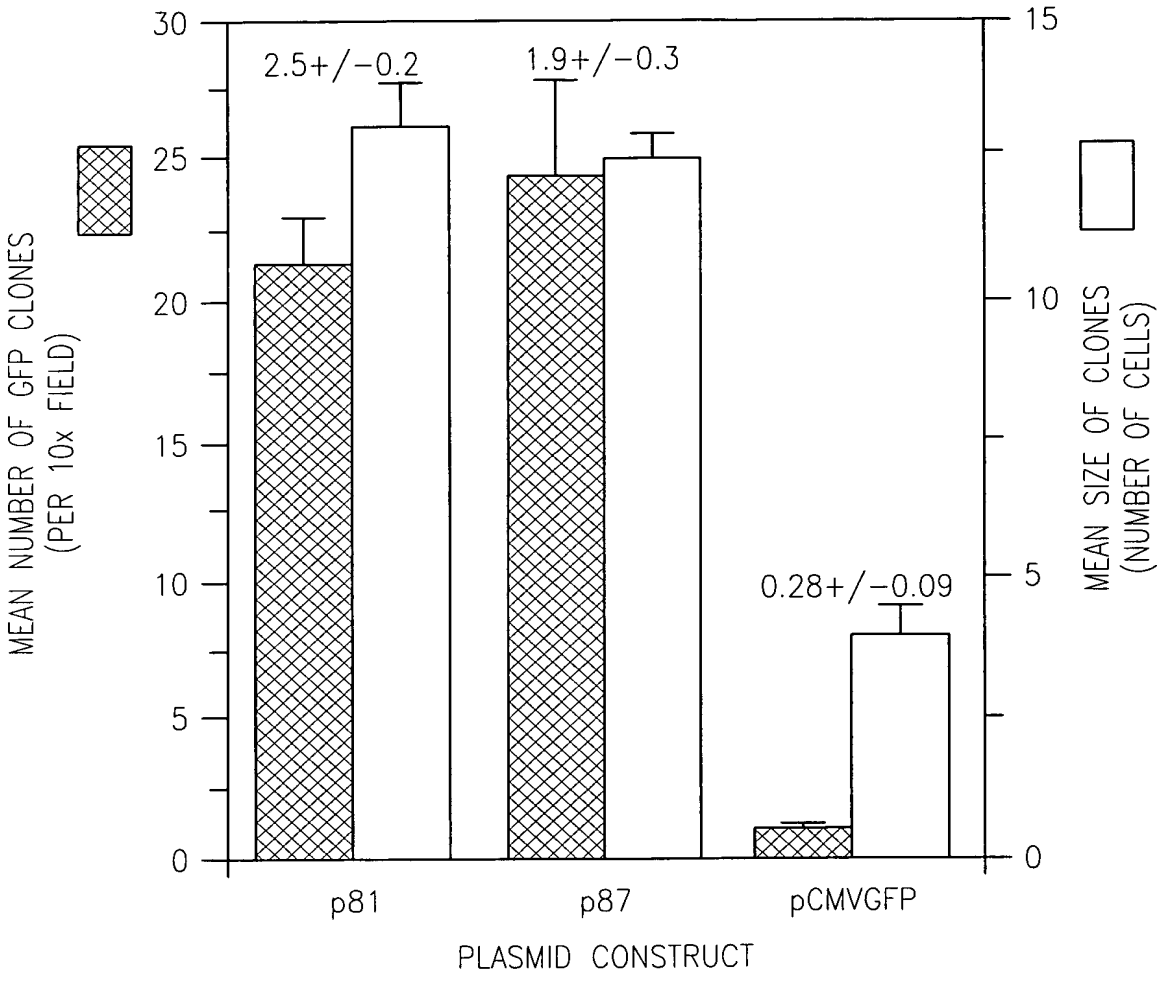

30
25
20
15
10
5
0
15
10
5
0
2.5+/−0.2
1.9+/−0.3
0.28+/−0.09
MEAN NUMBER OF GFP CLONES
(PER 10x FIELD)
MEAN SIZE OF CLONES
(NUMBER OF CELLS)
p81
p87
pCMVGFP
PLASMID CONSTRUCT

FIG. 7B

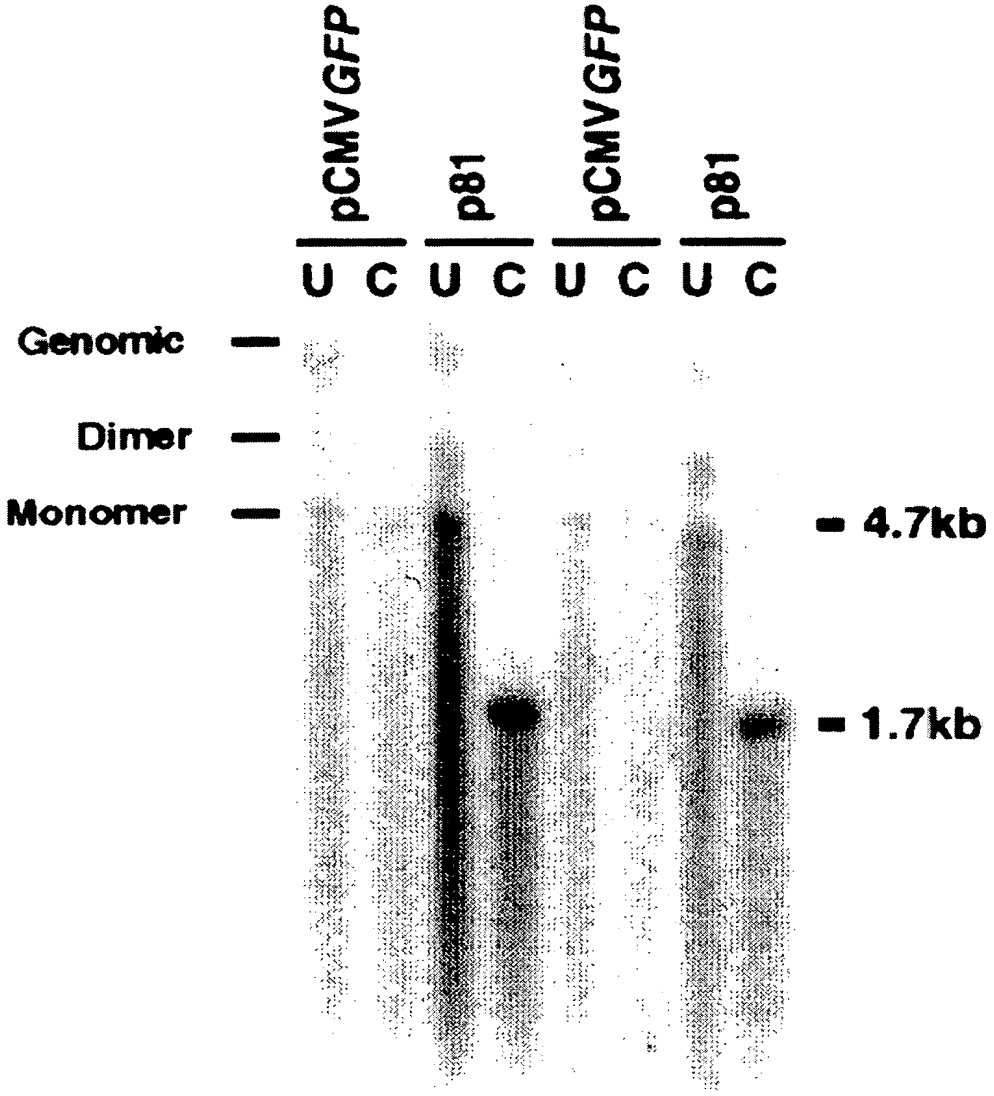

pCMVGFP
p81
pCMVGFP
p81
U C U C U C U C
Genomic
Dimer
Monomer
4.7kb
1.7kb

FIG. 7C

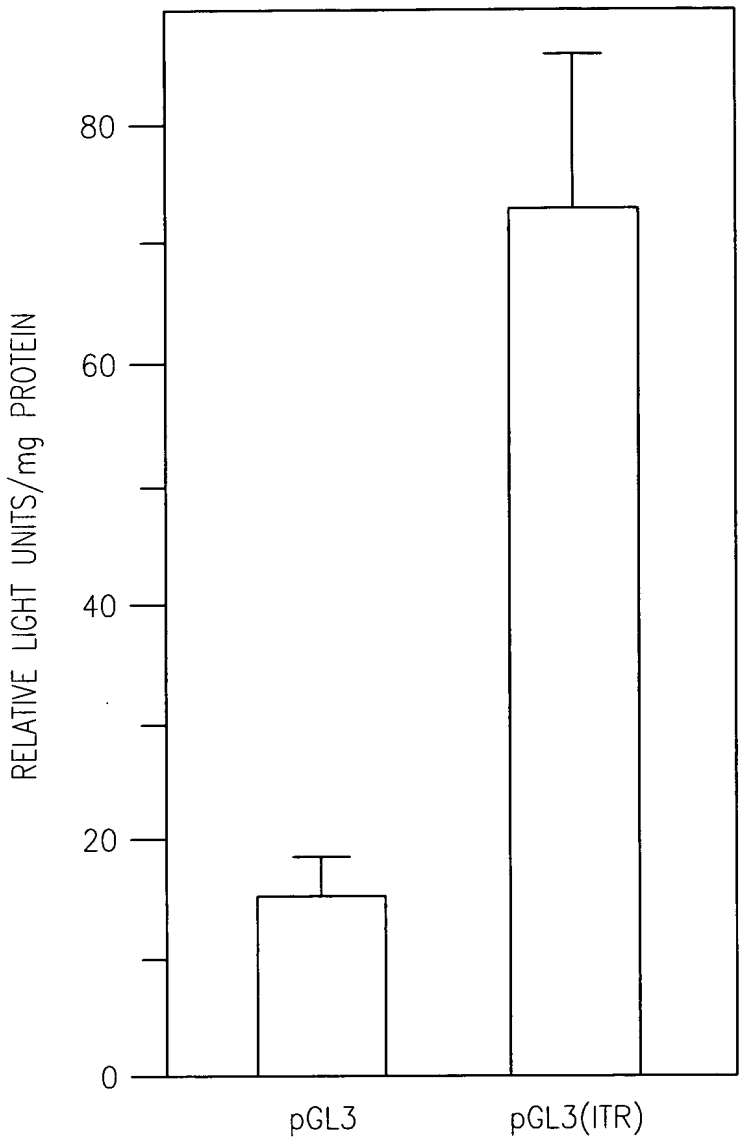



FIG. 7D

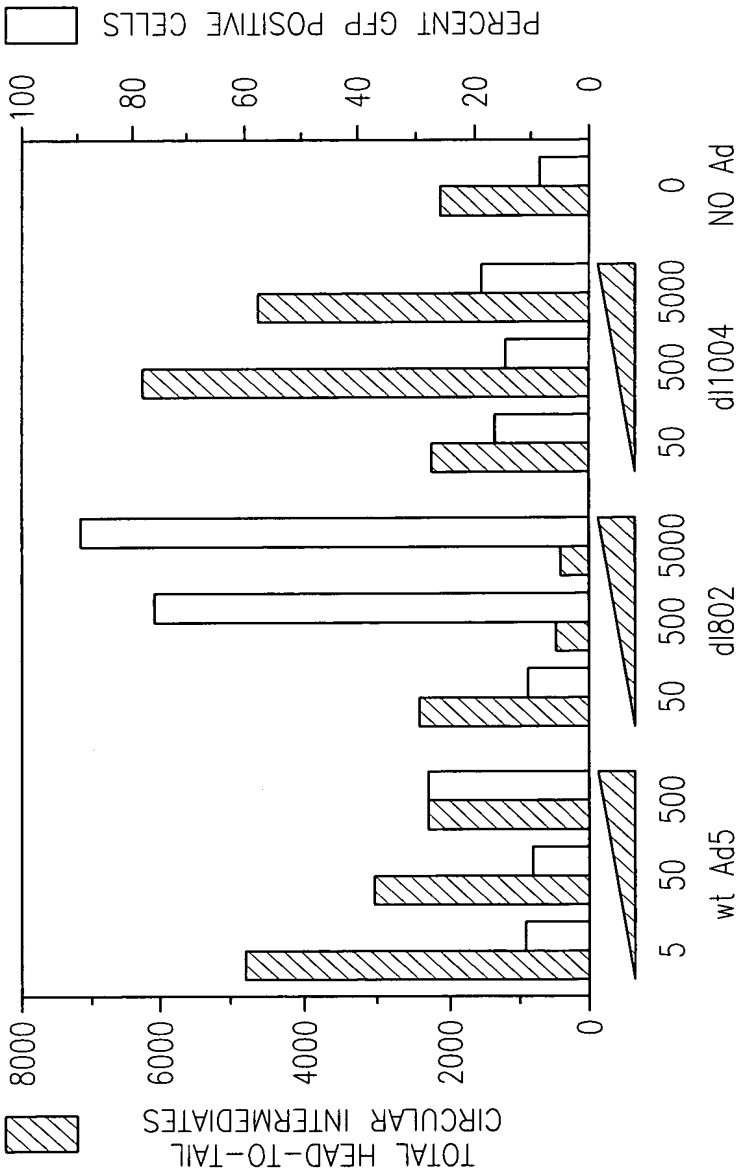

PERCENT GFP POSITIVE CELLS
100
80
60
40
20
0
8000
6000
4000
2000
0
TOTAL HEAD-TO-TAIL
CIRCULAR INTERMEDIATES
5
50
500
50
500
5000
50
500
5000
0
wt Ad5
dl802
dl1004
NO Ad

FIG. 8A

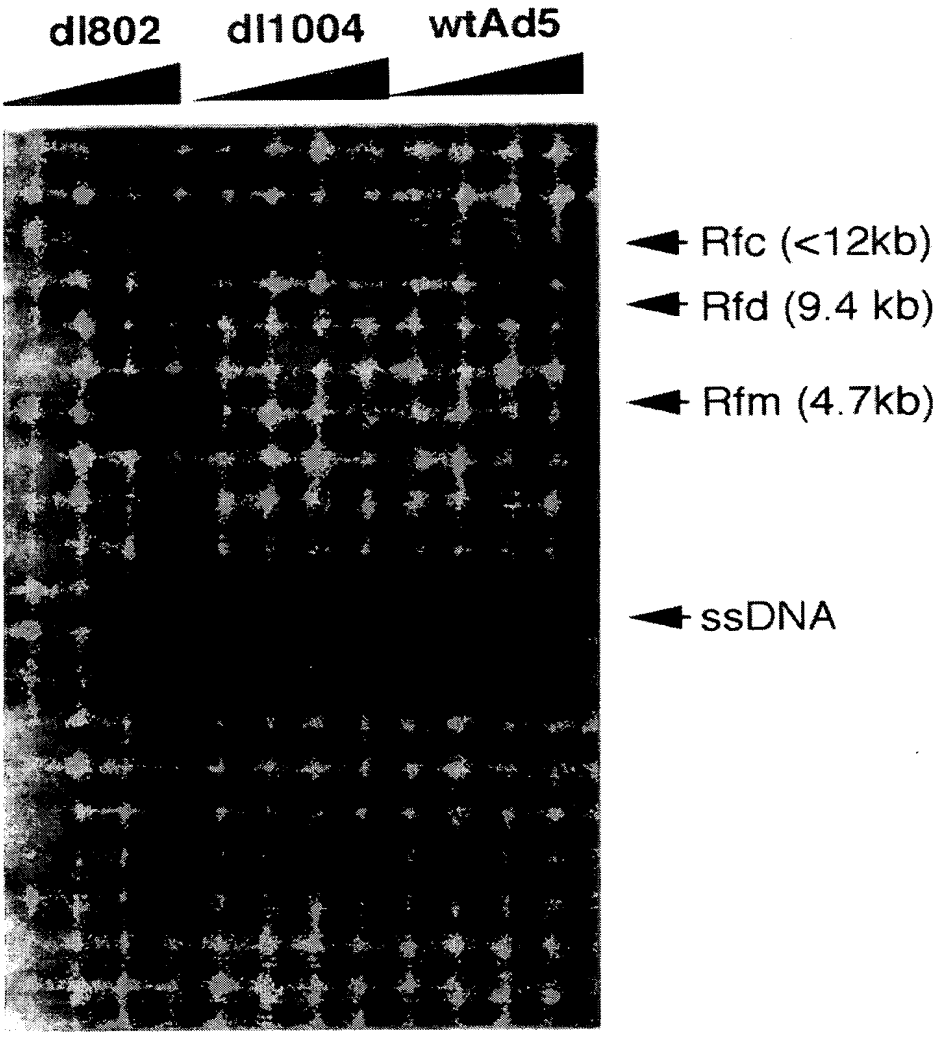



FIG. 8B

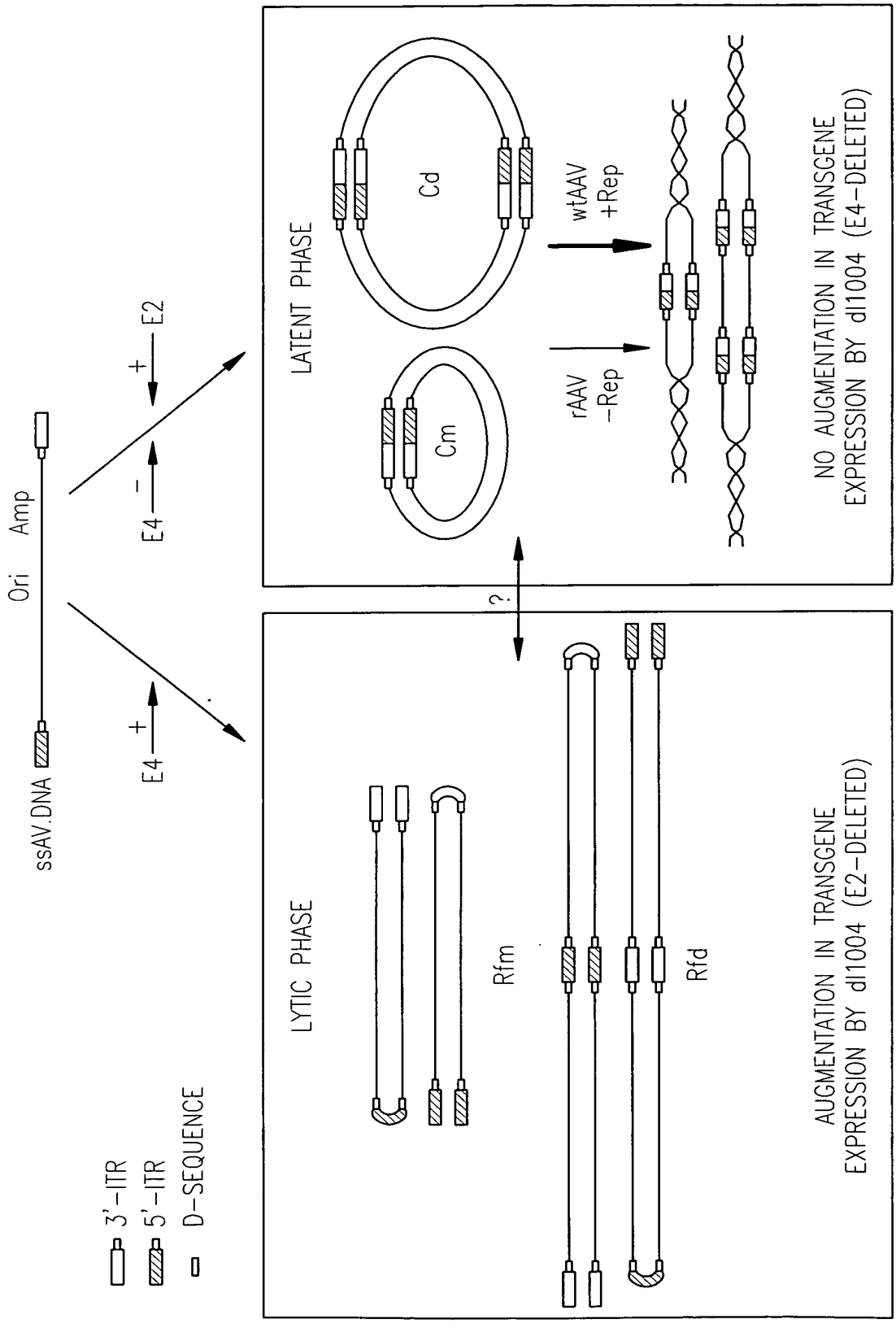

3'-ITR
5'-ITR
D-SEQUENCE
ssAV.DNA
Ori
Amp
E4
+
E4
−
E2
+
LYTIC PHASE
Rfm
Rfd
AUGMENTATION IN TRANSGENE
EXPRESSION BY dl1004 (E2-DELETED)
?
LATENT PHASE
Cd
Cm
rAAV
-Rep
wtAAV
+Rep
NO AUGMENTATION IN TRANSGENE
EXPRESSION BY dl1004 (E4-DELETED)

FIG. 9

```
        10         20         30         40         50         60
GCATGCAAGC TGTAGATAAG TAGCATGGCG GGTTAATCAT TAACTACAAG GAACCCCTAG
CGTACGTTCG ACATCTATTC ATCGTACCGC CCAATTAGTA ATTGATGTTC CTTGGGGATC

        70         80         90        100        110        120
TGATGGAGTT GGCCACTCCC TCTCTGCGCG CTCGCTCGCT CACTGAGGCC GGGCGGCCAA
ACTACCTCAA CCGGTGAGGG AGAGACGCGC GAGCGAGCGA GTGACTCCGG CCCGCCGGTT

       130        140        150        160        170        180
AGGTCGCCCG ACGCCCGGGC TTTGCCCGGG CGGCCTCAGT GAGCGAGCGA GCGCGCAGAG
TCCAGCGGGC TGCGGGCCCG AAACGGGCCC GCCGGAGTCA CTCGCTCGCT CGCGCGTCTC

       190        200        210        220        230        240
AGGGAGTGGC CAACTCCATC ACTAGGGGTT CCTTGTAGTT AATGATTAAC CCGCCATGCT
TCCCTCACCG GTTGAGGTAG TGATCCCCAA GGAACATCAA TTACTAATTG GGCGGTACGA

       250        260        270        280
ACTTATCTAC CGATGAATTC GAGCTTGCAT GC........
TGAATAGATG GCTACTTAAG CTCGAACGTA CG........
```

FIG. 10A

```
        10         20         30         40         50         60
GCATGCAAGC TGTAGATAAG TAGCATGGCG GGTTAATCAT TAACTACAAG GAACCCCTAG
CGTACGTTCG ACATCTATTC ATCGTACCGC CCAATTAGTA ATTGATGTTC CTTGGGGATC

        70         80         90        100        110        120
TGATGGAGTT GGCCACTCCC TCTCTGCGCG CTCGCTCGCT CACTGAGGCC GGGCGCGCGC
ACTACCTCAA CCGGTGAGGG AGAGACGCGC GAGCGAGCGA GTGACTCCGG CCCGCGCGCG

       130        140        150        160        170        180
TCGCTCGCTC ACTGAGGCCG GGCGACCAAA GGTCGCCCGA GCCCGGGCTT TGCCCGGGCG
AGCGAGCGAG TGACTCCGGC CCGCTGGTTT CCAGCGGGCT CGGGCCCGAA ACGGGCCCGC

       190        200        210        220        230        240
GCCTCAGTGA GCGAGCGCGC GCGCAGAGAG GGAGTGGCCA ACTCCATCAC TAGGGGTTCC
CGGAGTCACT CGCTCGCGCG CGCGTCTCTC CCTCACCGGT TGAGGTAGTG ATCCCCAAGG

       250        260        270        280        290        300
TTGTAGTTAA TGATTAACCC GCCATGCTAC TTATCTACCG ATGAATTCGA GCTTGCATGC
AACATCAATT ACTAATTGGG CGGTACGATG AATAGATGGC TACTTAAGCT CGAACGTACG
```

FIG. 10B

```
        10          20          30          40          50          60
GCATGCAAGC TGTAGATAAG TAGCATGGCG GGTTAATCAT TAACTACAAG GAACCCCTAG
CGTACGTTCG ACATCTATTC ATCGTACCGC CCAATTAGTA ATTGATGTTC CTTGGGGATC

        70          80          90         100         110         120
TGATGGAGTT GGCCACTCCC TCTCTGCGCG CTCGCTCGCT CACTGAGGCC GGGCGACCAA
ACTACCTCAA CCGGTGAGGG AGAGACGCGC GAGCGAGCGA GTGACTCCGG CCCGCTGGTT

       130         140         150         160         170         180
AGGTCGCCCG ACGCCCGGGC TTTGGTCGCC CGGCCTCAGT GAGCGAGCGA GCGCGCAGAG
TCCAGCGGGC TGCGGGCCCG AAACCAGCGG GCCGGAGTCA CTCGCTCGCT CGCGCGTCTC

       190         200         210         220         230         240
AGGGAGTGGC CAACTCCATC ACTAGGGGTT CCTTGTAGTT AATGATTAAC CCGCCATGCT
TCCCTCACCG GTTGAGGTAG TGATCCCCAA GGAACATCAA TTACTAATTG GGCGGTACGA

       250         260         270         280
ACTTATCTAC CGATGAATTC GAGCTTGCAT GC........
TGAATAGATG GCTACTTAAG CTCGAACGTA CG........
```

FIG. 10C

```
                10         20         30         40         50
P81       1 GCATGCAAGC TGTAGATAAG TAGCATGGCG GGTTAATCAT TAACTACAAG 50
p79       1 GCATGCAAGC TGTAGATAAG TAGCATGGCG GGTTAATCAT TAACTACAAG 50
p1202     1 GCATGCAAGC TGTAGATAAG TAGCATGGCG GGTTAATCAT TAACTACAAG 50

                60         70         80         90        100
P81      51 GAACCCCTAG TGATGGAGTT GGCCACTCCC TCTCTGCGCG CTCGCTCGCT 100
p79      51 GAACCCCTAG TGATGGAGTT GGCCACTCCC TCTCTGCGCG CTCGCTCGCT 100
p1202    51 GAACCCCTAG TGATGGAGTT GGCCACTCCC TCTCTGCGCG CTCGCTCGCT 100

               110        120        130        140        150
P81     101 CACTGAGGCC GGGCG----- ---------- ---------- ---GCCAAAG 150
p79     101 CACTGAGGCC GGGCGCGCGC TCGCTCGCTC ACTGAGGCCG GGCGaCcAAa 150
p1202   101 CACTGAGGCC GGGCG----- ---------- ---------- ---ACCAAAG 150

               160        170        180        190        200
P81     151 GTCGCCCGAC GCCCGGGCTT TGCCCGGGCG GCCTCAGTGA GCGAGCGAGC 200
p79     151 GgtcgCCcga GCCCGGGCTT TGCCCGGGCG GCCTCAGTGA GCGAGCGcGC 200
p1202   151 GTCGCCCGAC GCCCGGGCTT TGgtCGccCG GCCTCAGTGA GCGAGCGAGC 200

               210        220        230        240        250
P81     201 GCGCAGAGAG GGAGTGGCCA ACTCCATCAC TAGGGGTTCC TTGTAGTTAA 250
p79     201 GCGCAGAGAG GGAGTGGCCA ACTCCATCAC TAGGGGTTCC TTGTAGTTAA 250
p1202   201 GCGCAGAGAG GGAGTGGCCA ACTCCATCAC TAGGGGTTCC TTGTAGTTAA 250

               260        270        280        290        300
P81     251 TGATTAACCC GCCATGCTAC TTATCTACCG ATGAATTCGA GCTTGCATGC 300
p79     251 TGATTAACCC GCCATGCTAC TTATCTACCG ATGAATTCGA GCTTGCATGC 300
p1202   251 TGATTAACCC GCCATGCTAC TTATCTACCG ATGAATTCGA GCTTGCATGC 300
```

FIG. 11

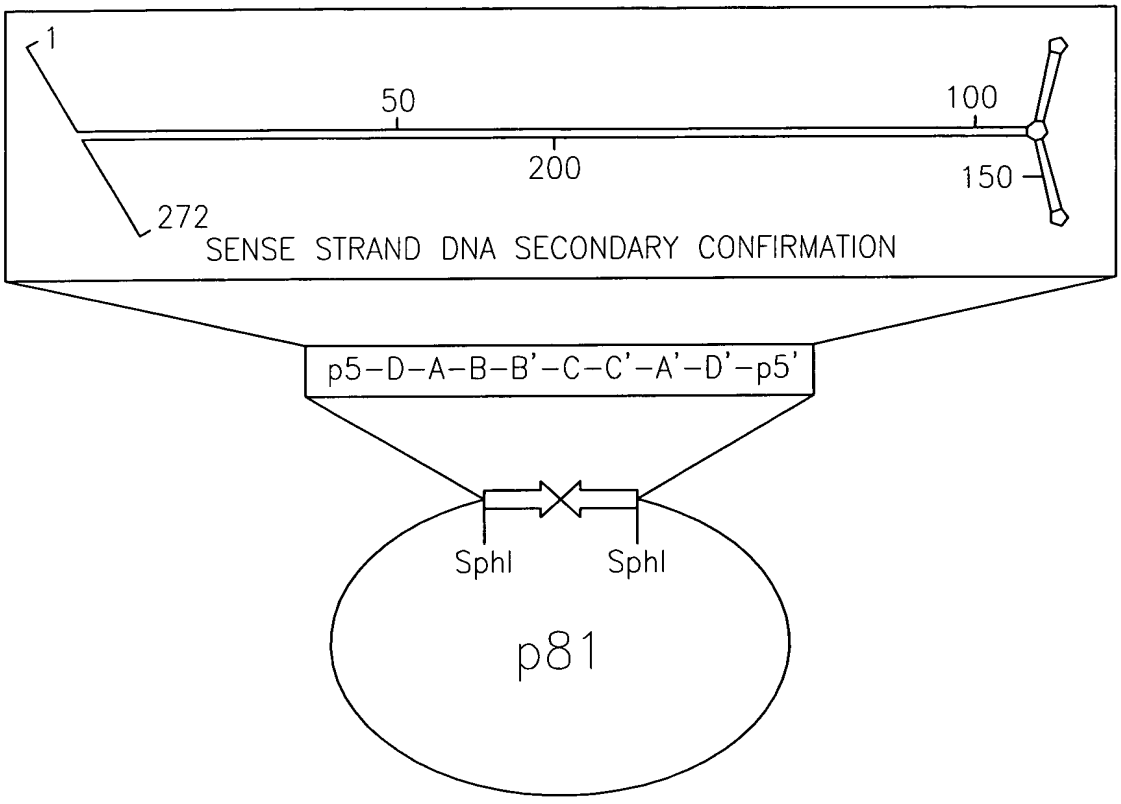

1
50
100
200
150
272
SENSE STRAND DNA SECONDARY CONFIRMATION
p5-D-A-B-B'-C-C'-A'-D'-p5'
SphI
SphI
p81

FIG. 12A

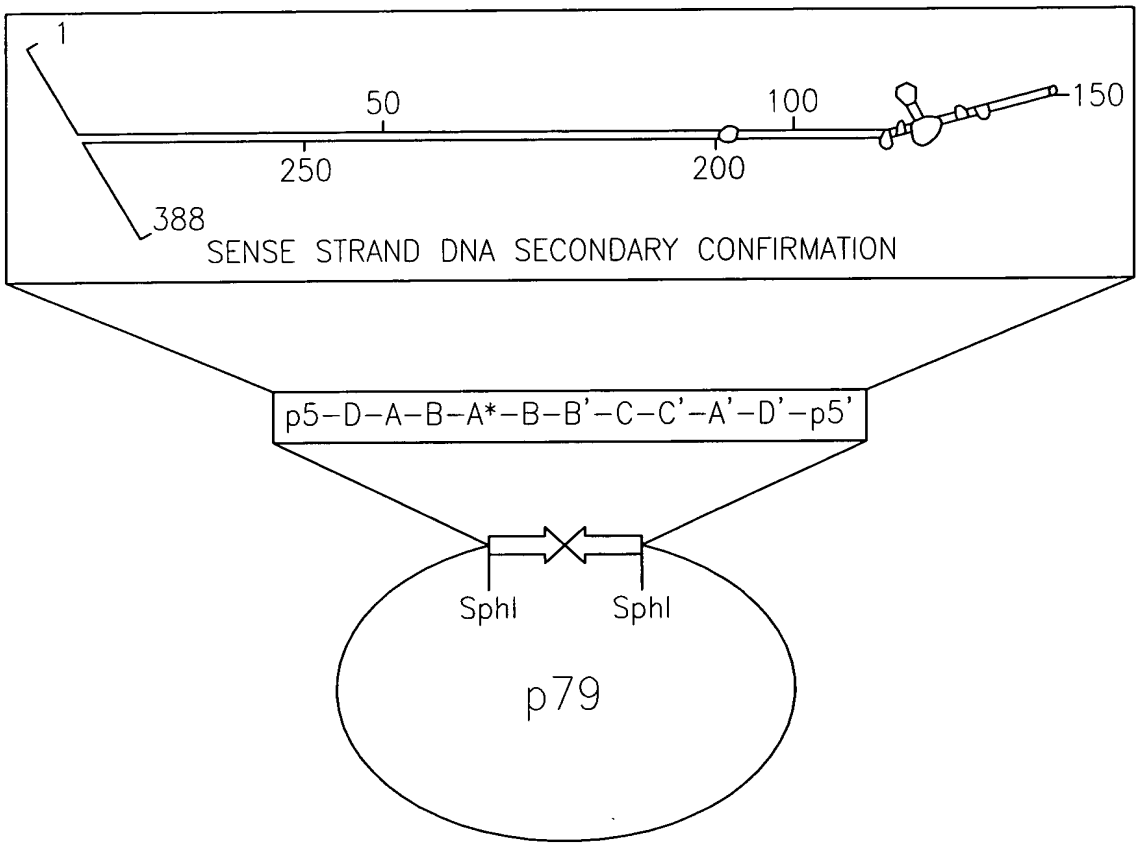

1
50
100
150
250
200
388
SENSE STRAND DNA SECONDARY CONFIRMATION
p5-D-A-B-A*-B-B'-C-C'-A'-D'-p5'
SphI
SphI
p79

FIG. 12B

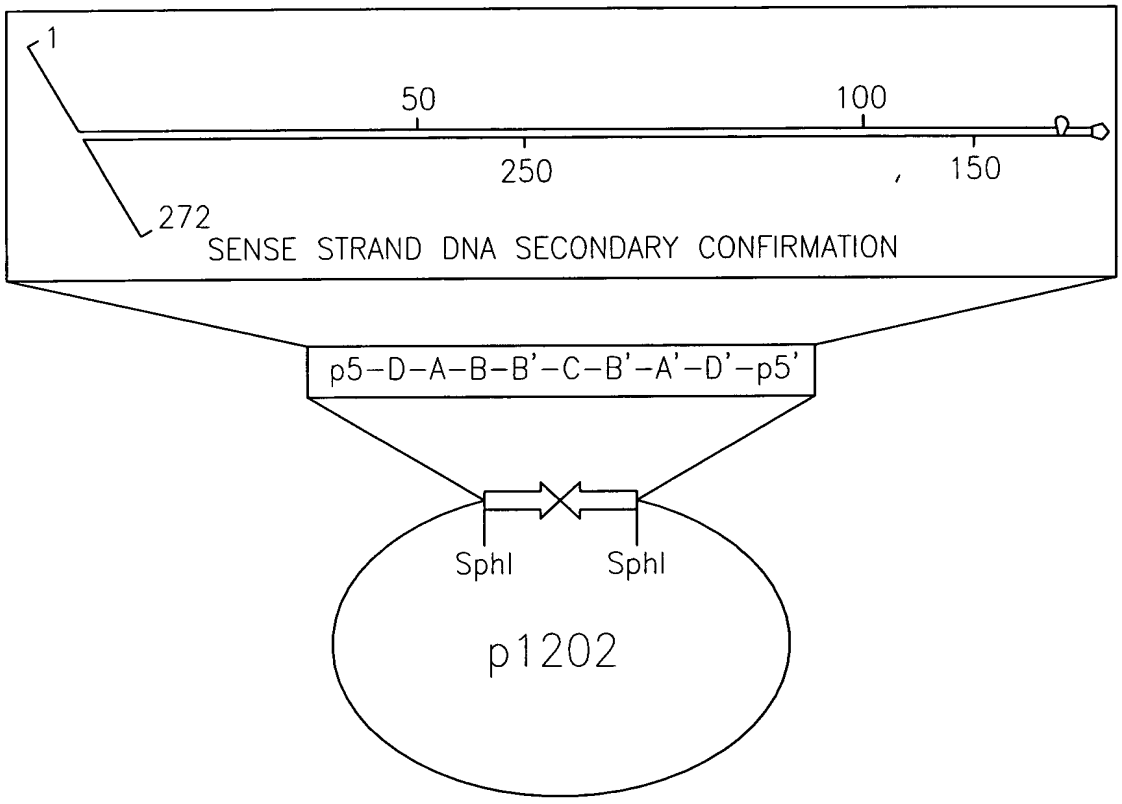

1
50
100
250
150
272
SENSE STRAND DNA SECONDARY CONFIRMATION
p5-D-A-B-B'-C-B'-A'-D'-p5'
SphI
SphI
p1202

FIG. 12C

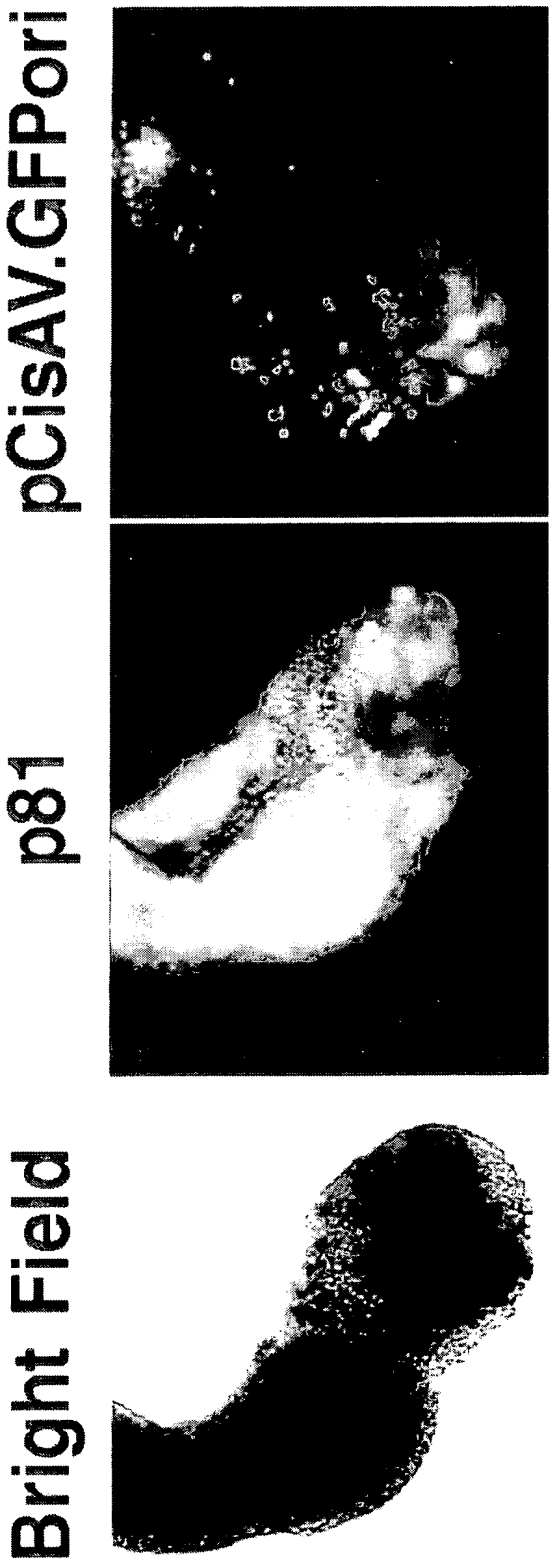



FIG. 13

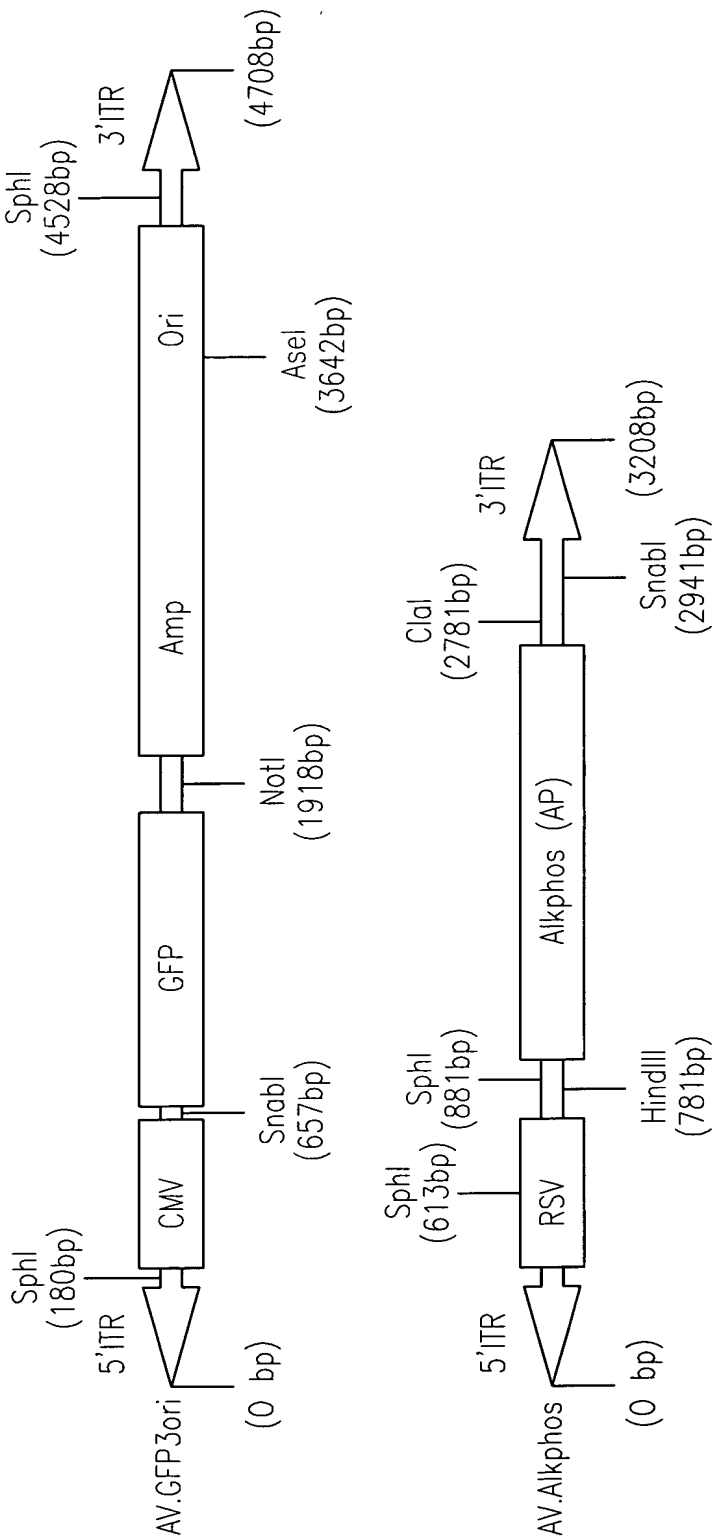

SphI
(180bp)
5'ITR
(0 bp)
AV.GFP3ori
CMV
GFP
SnaBI
(657bp)
NotI
(1918bp)
Amp
Ori
AseI
(3642bp)
SphI
(4528bp)
3'ITR
(4708bp)
SphI
(613bp)
5'ITR
(0 bp)
AV.Alkphos
RSV
Alkphos (AP)
SphI
(881bp)
HindIII
(781bp)
ClaI
(2781bp)
3'ITR
(3208bp)
SnaBI
(2941bp)

FIG. 14A

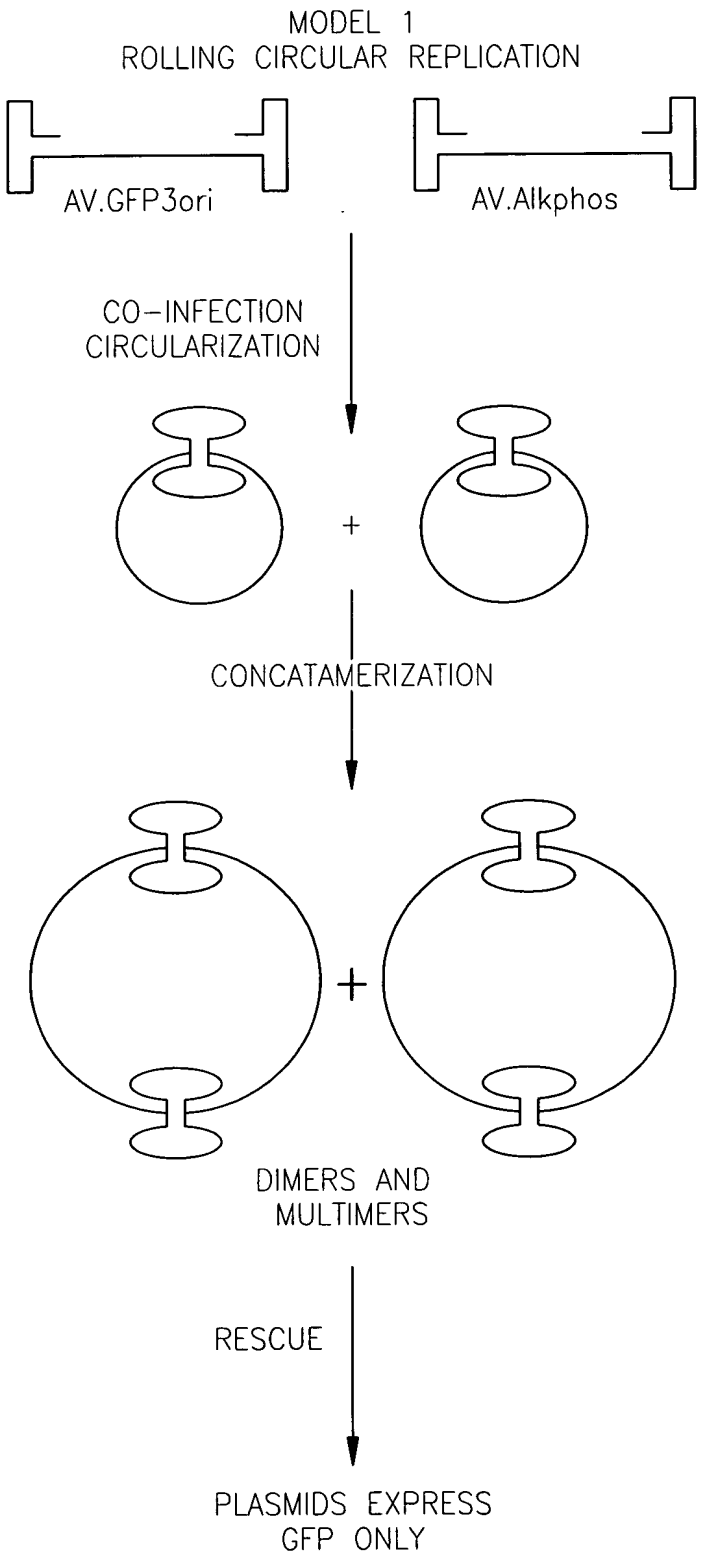

MODEL 1
ROLLING CIRCULAR REPLICATION
AV.GFP3ori
AV.Alkphos
CO-INFECTION
CIRCULARIZATION
+
CONCATAMERIZATION
+
DIMERS AND
MULTIMERS
RESCUE
PLASMIDS EXPRESS
GFP ONLY

FIG. 14B

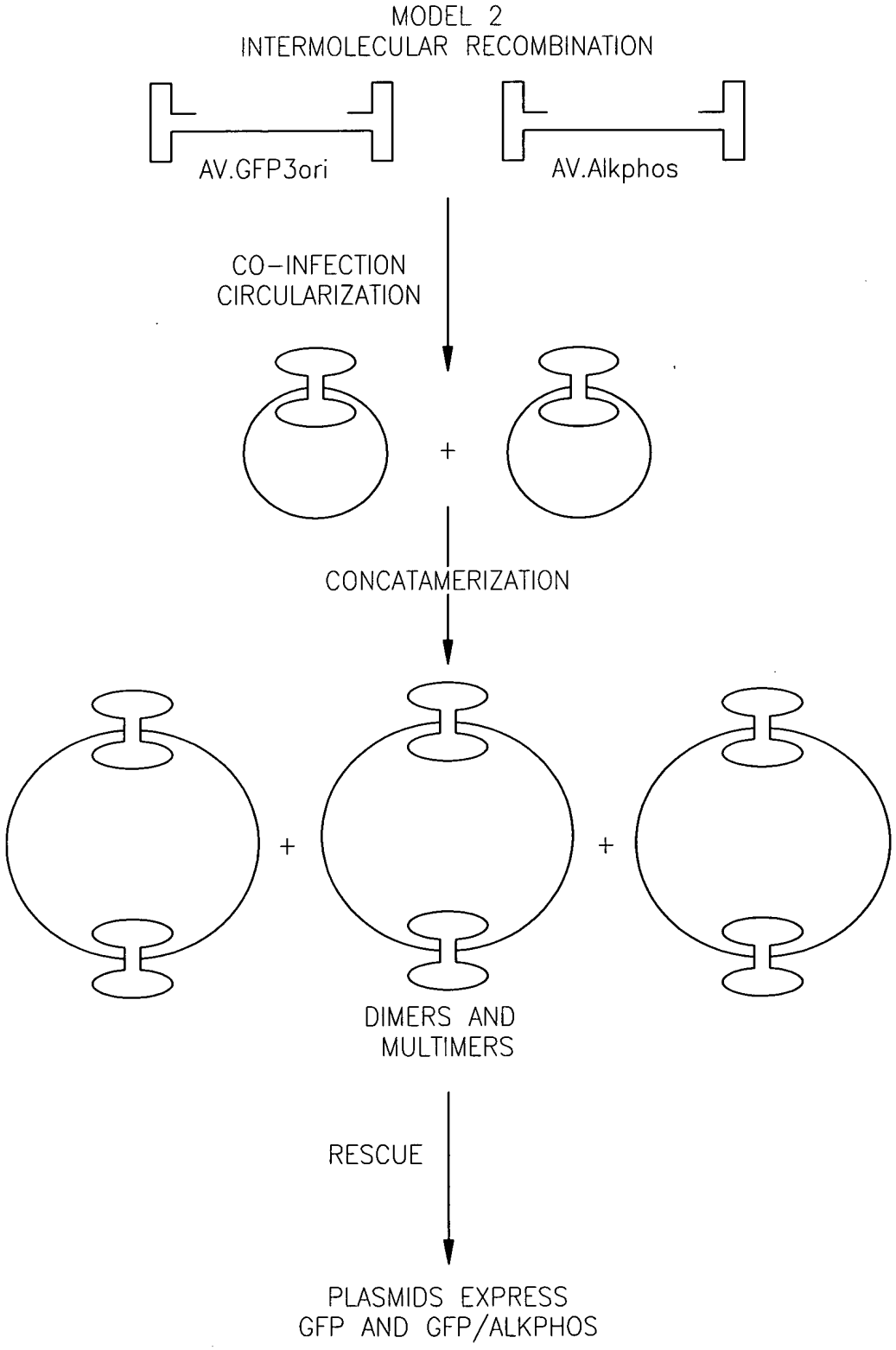

MODEL 2
INTERMOLECULAR RECOMBINATION
AV.GFP3ori
AV.Alkphos
CO-INFECTION
CIRCULARIZATION
+
CONCATAMERIZATION
+
+
DIMERS AND
MULTIMERS
RESCUE
PLASMIDS EXPRESS
GFP AND GFP/ALKPHOS

FIG. 14C

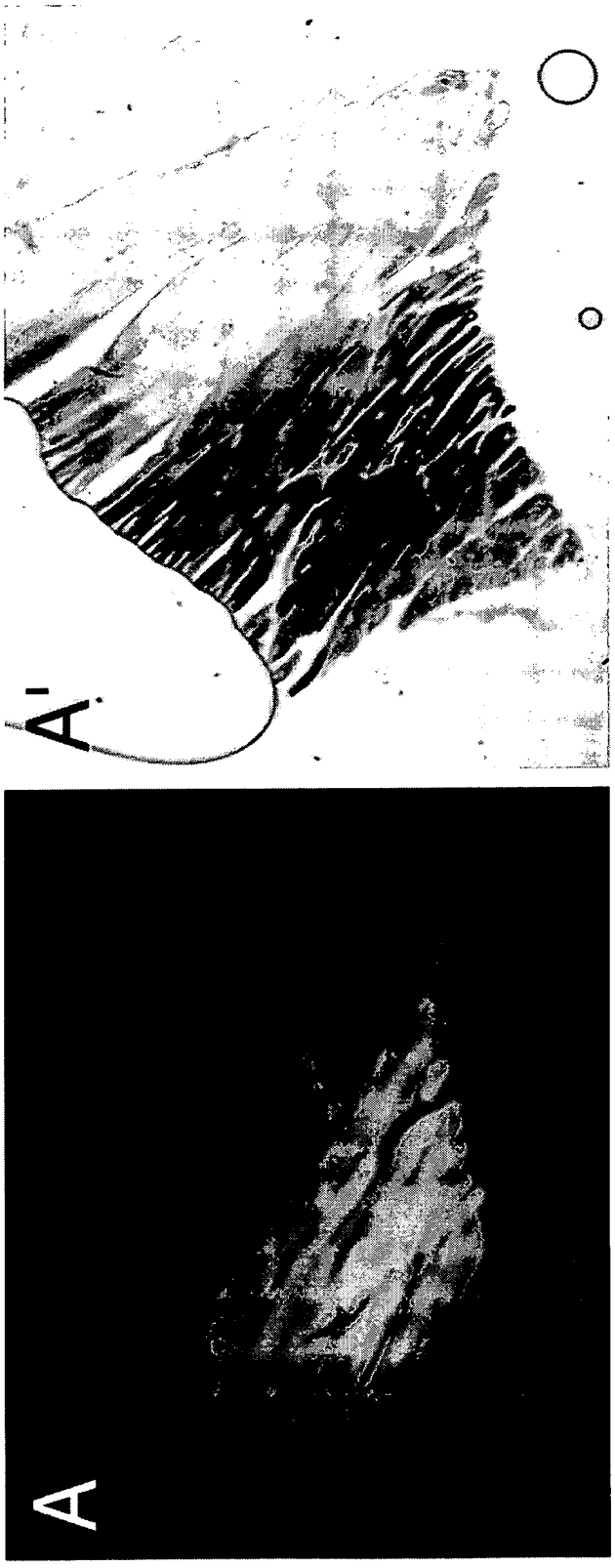
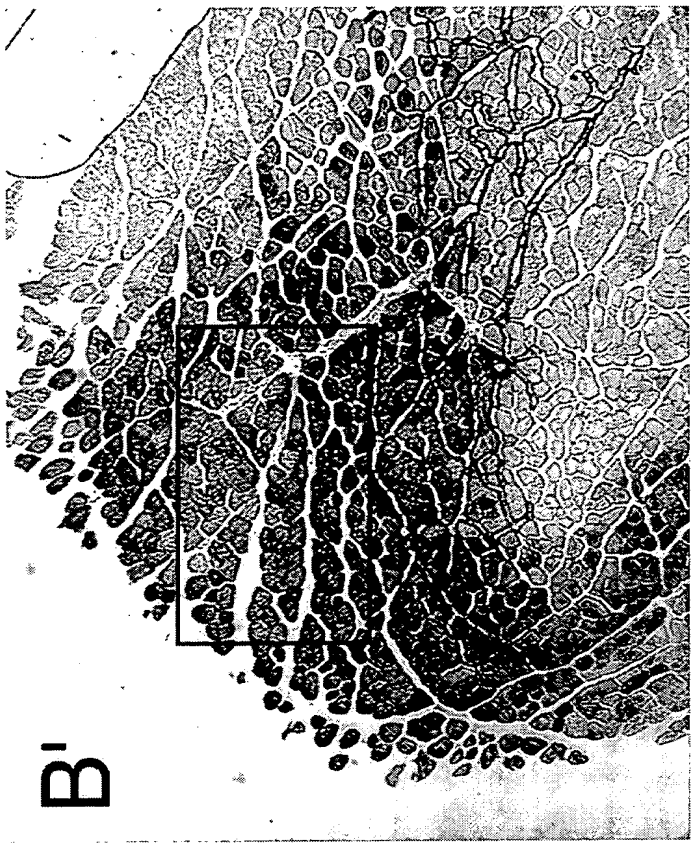



FIG. 15A



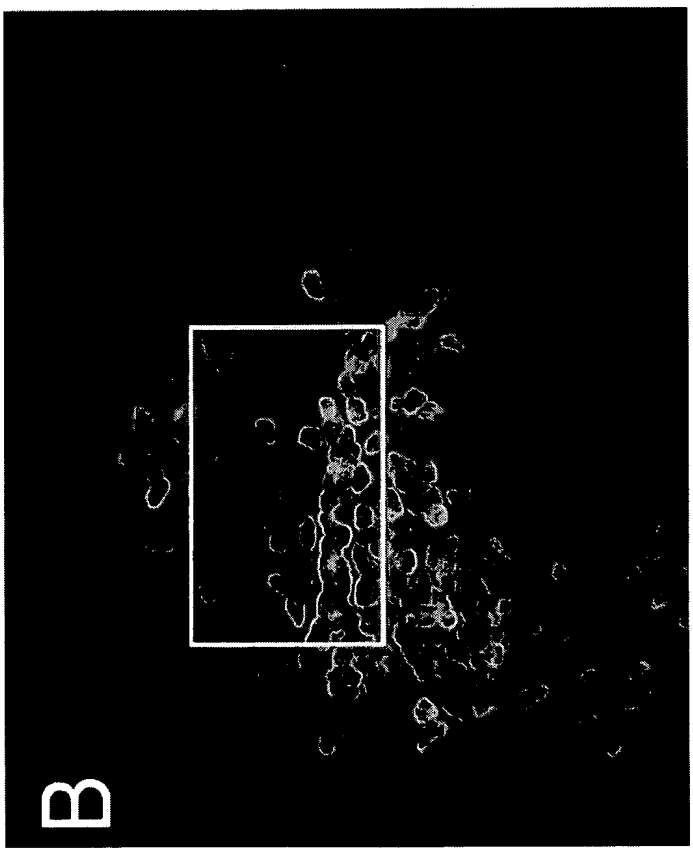




FIG. 15B

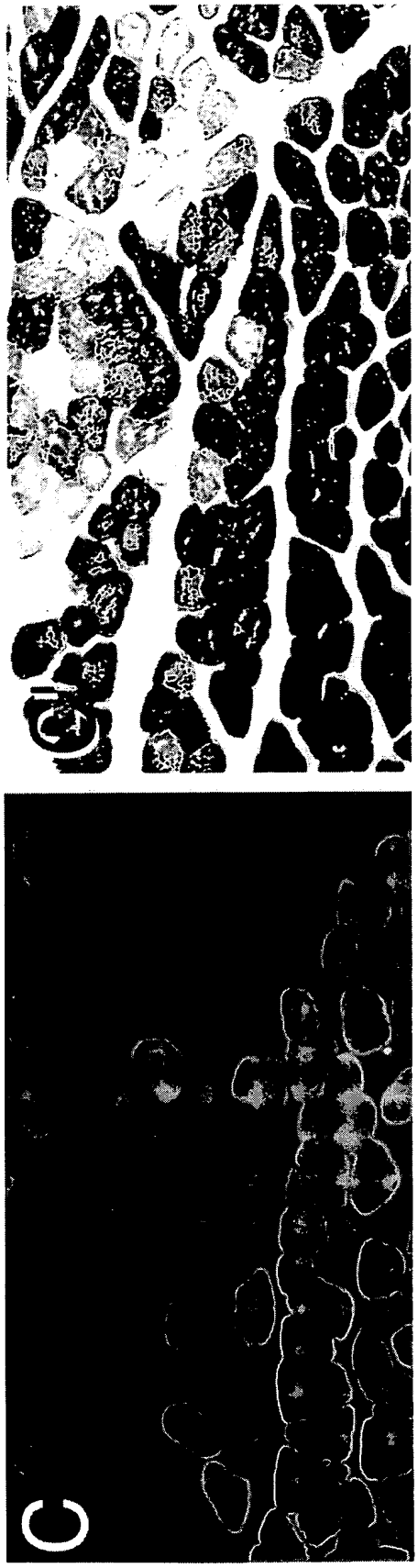



FIG. 15C

FIG. 15D

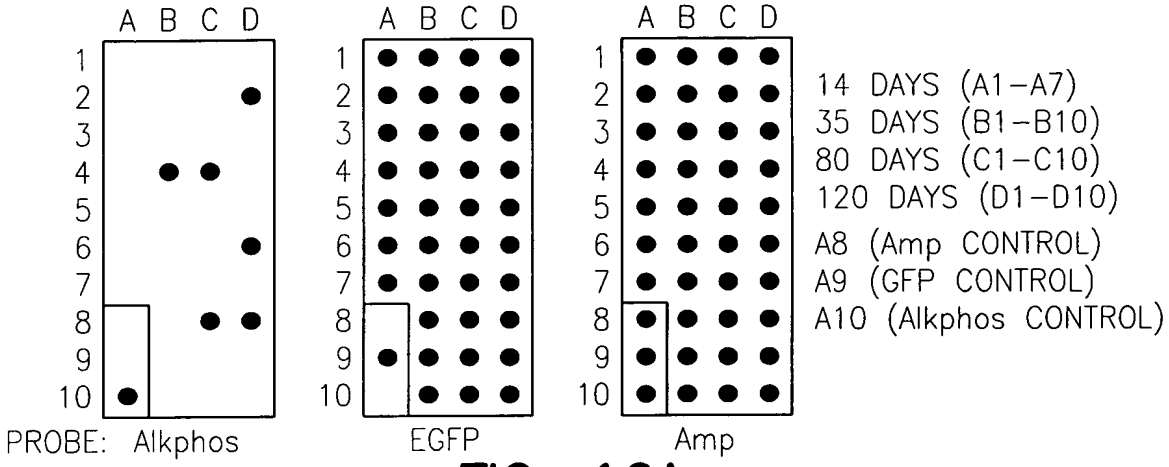



FIG. 16A

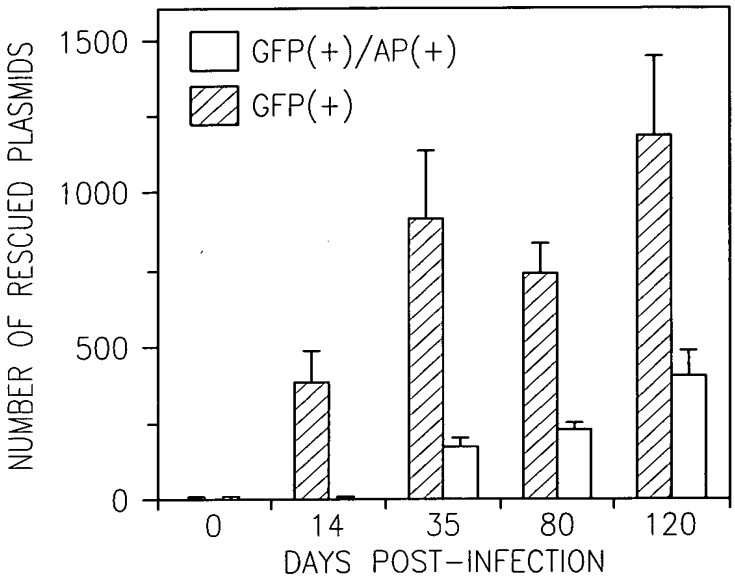



FIG. 16B

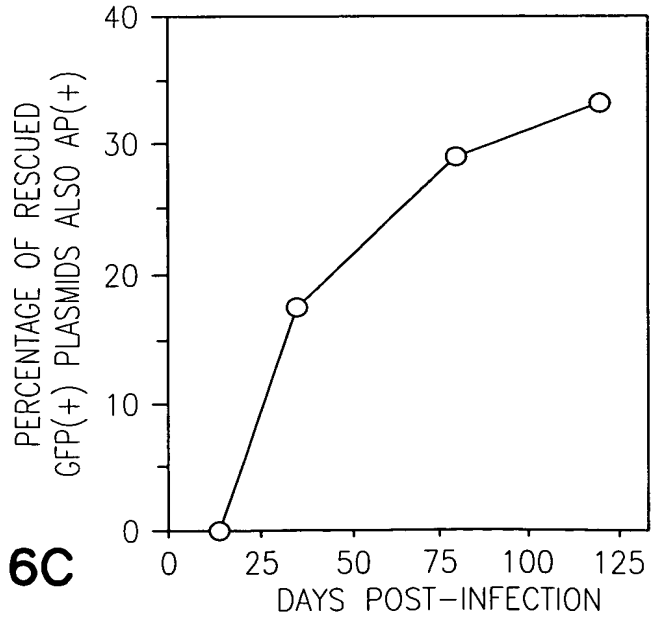
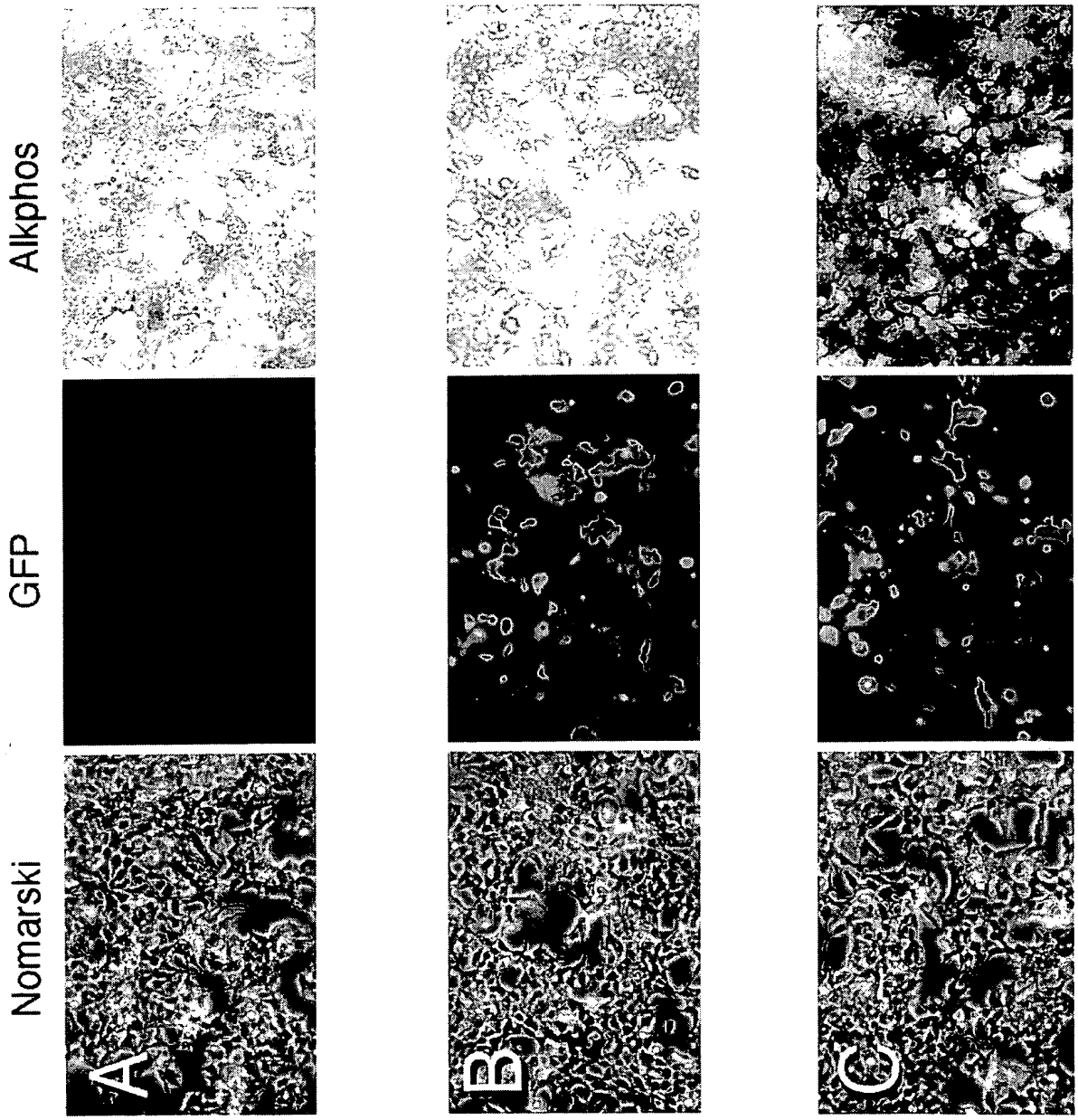



FIG. 16C

FIG. 17A

FIG. 17B

FIG. 17C



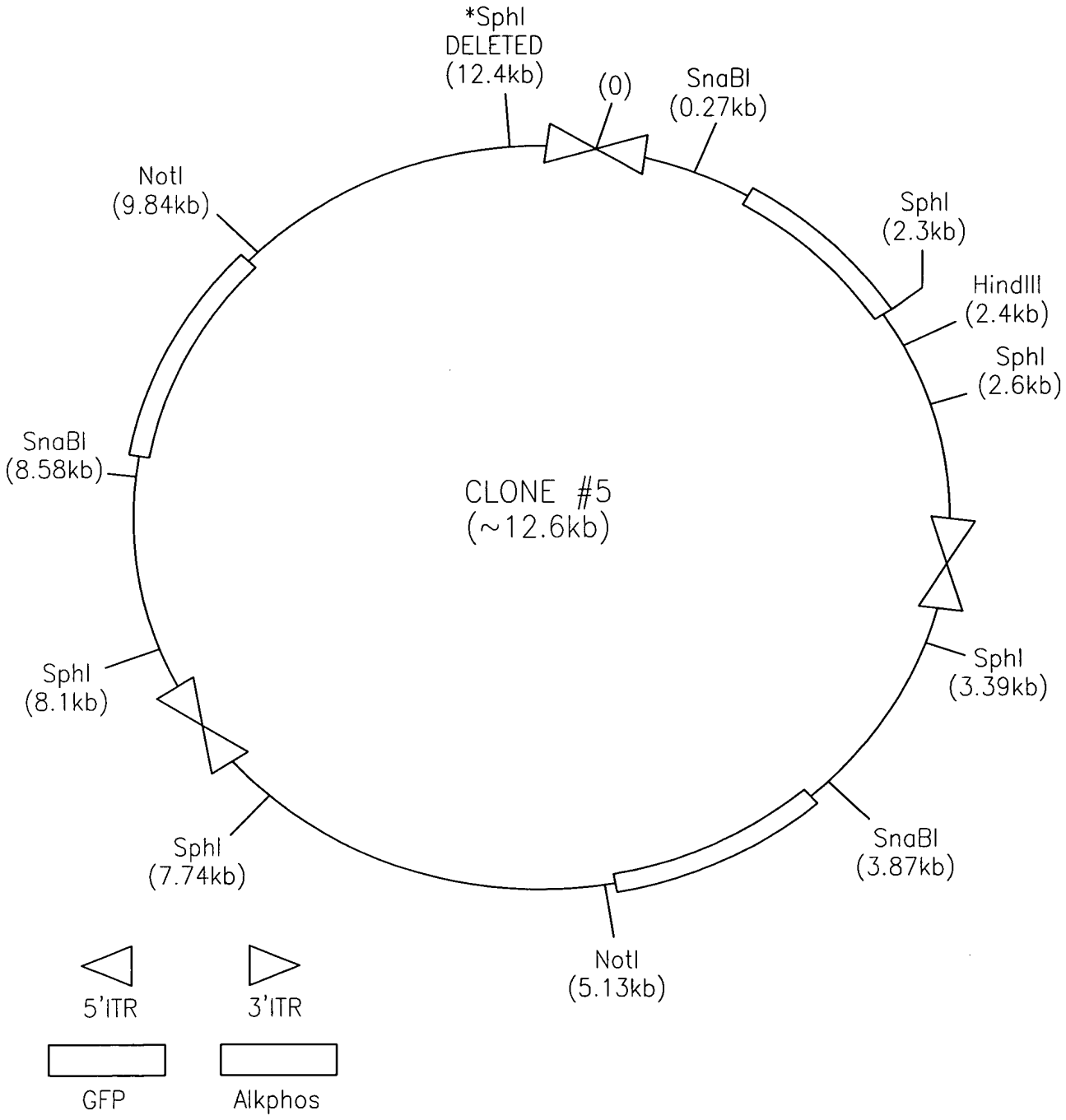

*SphI
DELETED
(12.4kb)
(0)
SnaBI
(0.27kb)
SphI
(2.3kb)
HindIII
(2.4kb)
SphI
(2.6kb)
SphI
(3.39kb)
SnaBI
(3.87kb)
NotI
(5.13kb)
SphI
(7.74kb)
SphI
(8.1kb)
SnaBI
(8.58kb)
NotI
(9.84kb)
CLONE #5
(~12.6kb)
5'ITR
3'ITR
GFP
Alkphos

FIG. 18D

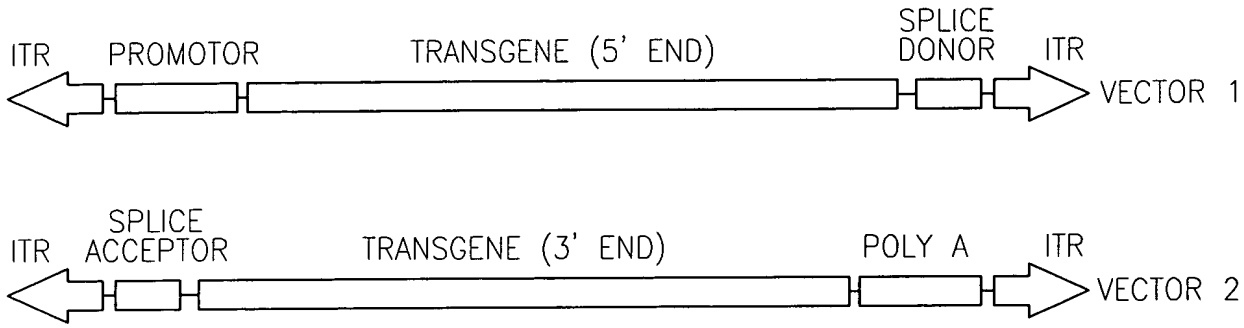



FIG. 19A

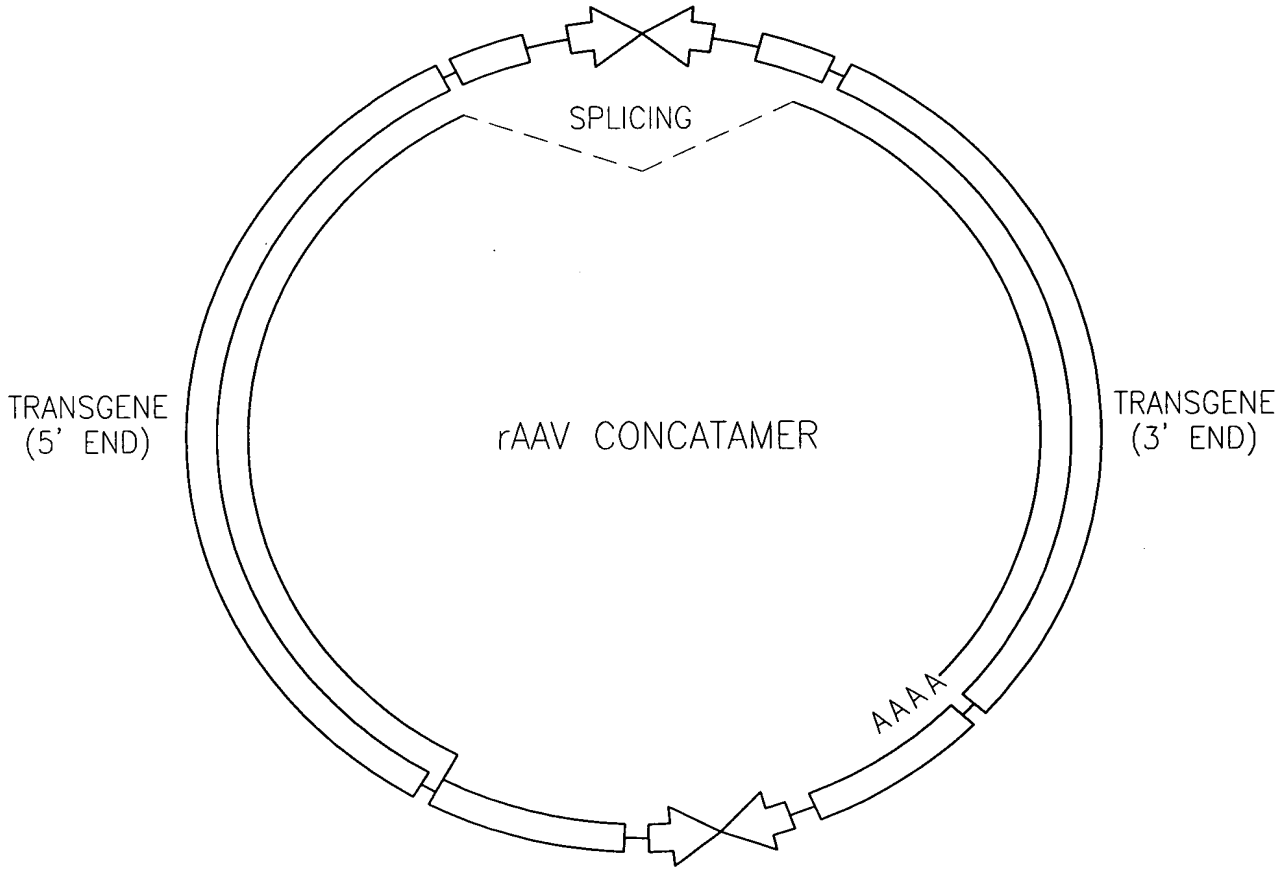



FIG. 19B

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